Understanding the habitat and decline of *Najas flexilis* (Willd.) Rostk. & Schmidt in the UK using ecology and paleoecology

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Declaration

I, Isabel Jane Bishop, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

Signature: Date:

Abstract

Najas flexilis is a rare macrophyte, protected under international legislation. This study aims to understand its contemporary decline in the UK, using a combination of spatial analyses and paleoecological reconstructions. Using a specially developed snorkel survey protocol, data on *N. flexilis* abundance, aquatic plant abundance, water depth, sediment characteristics, and basic water chemistry parameters were collected at 23 sites in Scotland. In combination with existing data, this showed that *N. flexilis* grew in different habitats with different vegetative communities in different lakes. Because *N. flexilis* relies on carbon dioxide for photosynthesis, alkalinity and competition from bicarbonate-utilising plants were influential variables. In acid-circumneutral sites, *N. flexilis* was found in low abundances, likely limited by acidity. In mildly alkaline, clear-water lakes, *N. flexilis* grew in high abundances. In more base-rich sites, *N. flexilis* was only found in the deepest reaches of the photic zone, below the minimum light tolerance levels of other plants. One significant competitor with *N. flexilis* was the invasive species *Elodea canadensis*, which can tolerate low light conditions and uses bicarbonate for photosynthesis.

Analyses suggested that *N. flexilis* is well represented in sediment cores. Historical records and macrofossil analyses suggest that *N. flexilis* has declined during the Anthropocene. Maximum abundances of *N. flexilis* macrofossils were associated with indicators of circumneutral-alkaline lakes with clear water. The decline of *N. flexilis* at Esthwaite Water aligned with the eutrophication of the site. At Loch of Craiglush, a more acid-circumneutral site, there was no evidence for nutrient enrichment within the lake, but all hydrologically connected sites had suffered from mild eutrophication. The loss of *N. flexilis* here also coincided with the expansion of *E. canadensis.* Upon consideration of both the ecological and paleoecological investigations, it is concluded that the biggest threats to *N. flexilis* in the UK are eutrophication and invasive species invasion.

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1. Introduction

1.1 Freshwater ecosystems

For millennia, humans have relied on freshwater ecosystems to provide a wide range of services, including drinking water, irrigation, transportation, harvest of plants, fish and materials, and as sites for settlement (Strayer and Dudgeon, 2010). Freshwater ecosystems are estimated to contribute 20% of the economic value of all ecosystems on Earth (Constanza *et al.*, 1997). Many of these ecosystem services are the result of the extraordinary ecological diversity supported by freshwater environments. For example, they support 9.5% of all described animal species in the world, despite only covering 0.8% of the Earth's surface area (Balian *et al.*, 2008). However, freshwater ecosystems are suffering biodiversity loss at far greater rates than terrestrial ecosystems due to overexploitation, water pollution, flow modification, habitat destruction and degradation and invasion by exotic species (Dudgeon *et al.*, 2006). Aquatic macrophytes (i.e. submerged, emergent, free-floating or floating-leaved plants that grow on or near water) have been particularly affected, with declines documented worldwide as a result of anthropogenic alterations to lakes, rivers and estuaries (Dennison *et al.*, 1993). In healthy lake ecosystems, aquatic macrophytes play an important role as primary producers, as habitat for benthic algae, invertebrates and fish, and in nutrient and carbon cycling (Carpenter and Lodge, 1986). By providing habitat for algal-grazing fauna, macrophytes provide resilience to ecosystem-wide shifts towards algal dominance in cases of nutrient enrichment (Jeppesen *et al.*, 1998). Despite providing such an important role within freshwater ecosystems, macrophytes are particularly vulnerable to the threats currently faced by these fragile environments (Chambers *et al.*, 2008).

1.2 Importance of rare species

"Rare" species (i.e. species that have low abundance and/or small geographical ranges (Gaston, 1994)) play a valuable role in lake ecosystem functioning. They make an

important contribution to species diversity, which has repeatedly been shown to increase a habitat's resilience to eutrophication (Jeppesen *et al.*, 2000) and species invasion (Lyons and Schwartz, 2001; Van Ruijven *et al.*, 2003). Rare species can also play a disproportional role in delivering ecosystem services; for example, in Alaska's Copper River Delta, uncommon *Equisitum* spp. have been demonstrated to use their deep roots to sequester nutrients from the lower C horizons to the upper O horizons of the soil, contributing as much as 29% of total phosphorus in the nutrient cycling of the wetland (Marsh *et al.*, 2000). By applying a functional ecology approach to large datasets of coral reef fishes, alpine plants, and tropical trees, Mouillot *et al.* (2013) showed that rare and uncommon species have traits which allow them to fulfill functions not supported by other species, and that loss of rare species therefore leads to loss of functional diversity. In impacted environments, rare species are often the first to disappear. Because the specific contribution of many rare species to ecosystem services and functioning is poorly understood, a precautionary approach to their protection is often advocated (Lyons *et al.*, 2005). Furthermore, the presence of rare species can be an indicator of overall ecosystem health. Examining freshwater fish, birds, mammals, freshwater mussels, reptiles and amphibians in the Middle Atlantic region of the United States, Lawler *et al.* (2003) showed that habitats containing one rare species are more likely to contain another rare species from a different group, and that rare species can therefore be used as a biodiversity indicator. Cao *et al.* (1998) showed that exclusion of rare species from bioassessments of benthic invertebrates of the River Trent, England, was found to have a significantly greater impact on the survey results of sites with good water quality than on results from sites with poor water quality. Since preventing biodiversity loss is a central concern of modern conservation, both the preservation and monitoring of rare or uncommon species are key (Loreau *et al.* 2001).

1.3 Species conservation and legislation

Because of their importance, rare freshwater macrophytes draw legal protection under both international and domestic legislation. The Convention on the Conservation of

European Wildlife and Natural Habitats ("The Bern Convention") came into effect in 1981, aiming to ensure conservation of wild flora and fauna and their habitats with a special focus on endangered and vulnerable species (Council of Europe, 1979). The UK responded by introducing the Wildlife and Countryside Act (1981), which, alongside enhanced protection for designated Sites of Special Scientific Interest (SSSIs), called specifically for the conservation of a list of rare species including seven of the UK's aquatic macrophytes. The United Nations Conference on Environment and Development, held in Rio de Janeiro in 1992, bought global attention to the issue of biodiversity loss, and led to the creation of the Convention on Biological Diversity (CBD). To date, 195 states have agreed to implement national biodiversity strategies and action plants in accordance with this treaty. A 196th signatory - the European Union - has created a continental-scale strategy to tackle biodiversity loss. The EU Habitats Directive created a legal obligation for all member nations to protect the habitats and species specifically listed in the various annexes of the document (Council of the European Union, 1992). In response to both the CBD and the EU habitats Directive, the UK introduced the UK Biodiversity Action Plan (UKBAP) in 1994 (HMSO, 1994). This legislation provides detailed plans for the conservation of all habitats and species listed in the Habitats Directive, including two of the UK's rarest aquatic plants; *Luronium natans* and *Najas flexilis*. It requires all protected areas to be returned to, or maintained at, "favourable conservation status". For sites which are known to contain *L. natans* or *N. flexilis,* the presence of healthy populations of the protected plant is an essential criterion for achieving "favourable conservation status".

1.4 Challenges in rare species conservation

Consistent, reliable documentary records and ongoing monitoring both play an important role in informing the conservation of rare species. Documentary records are essential to understanding the natural history of the species, locating suitable habitats, and providing a framework for assessing modern patterns and processes (Swetnam *et al.*, 1999). However, long term records of the changing presence and abundance of rare aquatic

species through time are often lacking (Dudgeon *et al.*, 2006). This is exacerbated by the fact that species are often mis-identified, leading to species being either falsely recorded or omitted from historical records (Morrison *et al.*, 1998). Museum records in particular tend to be sparse, and often suffer from spatial bias (Rondinini *et al.* 2006). Sutherland *et al.* (2004) argue that the paucity of historical data currently forces many conservation decisions to be based on anecdotal evidence, equating current environmental practices to those common in healthcare before the introduction of "evidence-based practice". In order to carry out effective, evidence-based conservation, this data gap must be urgently addressed. While documentary records are vital for providing historical context, ongoing, accurate, reliable and repeatable measuring and monitoring is the only way to evaluate the changing status of rare species. Rare species are, by definition, more difficult to survey than commonplace species. Rare species have a low detection probability, meaning that a large proportion of random sampling sites are unlikely to shelter the species (Pollock *et al.*, 2004). Typically, rare species monitoring programs therefore have a strong focus on survey effort towards positively locating, identifying and recording the target species. However, the elimination of the random component in sampling strategies can introduce bias that affects the quantification of the realised niche of the species, impacting the conclusions drawn (Guisan *et al.*, 2006). Rare aquatic plants present a particular challenge because they are often not readily visible from above the water surface, and locating them requires special equipment including boats, bathyscopes, grapnels and sometimes diving equipment. Until recently, there was no standard methodology for the deployment of these techniques, and sampling strategies varied greatly between different studies (Gunn *et al.*, 2010).

The introduction of the EU Habitats Directive and the UKBAP have started to address the gap in available data on rare species, since they rely on accurate, reliable and repeatable measuring and monitoring for the evaluation of "ecological status". Under the EU Habitats Directive, priority habitats and species must be monitored regularly. In the case of freshwater habitats, monitoring must take place every six years at a minimum (Council of the European Union, 1992). These monitoring programs follow a standard methodology, and are referred to as Site Condition Monitoring in Scotland and Common Standards Monitoring in the rest of the UK. Here, they will be referred to as Site

Condition Monitoring, abbreviated to SCM. SCM methods are designed to monitor ecological communities and habitats, but are supplemented with more focussed surveys that specifically target rare species. Despite the importance of rare species assessments, there is little guidance available for the methodologies that should be employed during these surveys. As a result, rare species monitoring programs implemented since 2000 have used a variety of survey techniques and sampling strategies. Nevertheless, SCMs have produced large datasets on rare aquatic macrophytes and the habitats that they occupy that span multiple locations across a period of nearly twenty years (being first implemented in 2000). These datasets have the potential to reveal important information on the ecology and changing status of rare macrophytes, which could be used to aid their conservation in the future.

1.5 Paleoecology as a solution to the "data gap"

SCM data has gone some way to addressing data gaps on rare species over the past twenty years and should continue to do so into the future. In recent years, a growing number of studies have applied approaches of Quaternary paleolimnology to try to fill in data gaps from before 2000. The field of 'applied paleoecology' addresses specific ecological questions typically spanning the past 100 – 200 years; the time scale most relevant to nature conservation. Paleoecology is the study of the ecology of the past using evidence gathered from fossils (e.g. pollen, seeds, insect exoskeletons and diatom frustules) preserved in sediments, often in combination with physical, chemical, biogeochemical and stable-isotope analyses of the sediments themselves (Birks, 2008). A review by Birks (1996) outlines two ways in which paleoecology can contribute specifically to the conservation of rare species; a) through direct reconstruction of population changes of the species in question, and b) through assessment of habitat decline. The first is illustrated through the example of the aquatic macrophytes *Najas marina, Hydrocharis morsus-ranae, Potamogeton friesii, Potamogeton compressus, Potamogeton obtusifolus* and *Stratiotes aliodes* in the Norfolk Broads, which, through fossil reconstructions, were shown to have declined in response to nutrient enrichment

(Jackson, 1978). Another example is the bryozoan *Lophopus crystallinus*, whose known UK distribution increased more than 10-fold following counts of statoblasts in lake sediments (Hill *et al.*, 2007). The second is demonstrated in the case of the Natterjack Toad *Bufo calamita;* diatoms, macrofossils, heavy metals and soot particles in sediment cores show that breeding pools in southern England had been subject to acidification, and that this was likely responsible for the decline of the species (Beebee *et al.*, 1990).

Rare macrophytes, specifically, can be directly studied as fossil plant remains ("macrofossils") laid down in lake sediments. Ecological theory states that the presence of a plant at any location depends upon the ability of the species to a) disperse and b) colonise that site (MacArthur and Wilson 1967). The distribution of the reproductive parts of plants that are laid down in the sediment and later analysed by paleolimnologists as macrofossils represent the dispersal stage of this process. However, different plant species produce and transport remains in different ways, and this can introduce bias in the interpretation of macrofossil reconstructions. Birks (1973) critically analysed this relationship for all wetland and aquatic plants found in 32 lakes in Minnesota. For all plants, the distribution of seed macrofossils was related to the dispersal strategy of the plant – for example, seed macrofossils of *Ranunculus sceleratus*, whose ovules float on water, were found predominantly in areas of open water. In general, macrofossils of obligate aquatic plants were only found in small areas located close to parent plants; this was attributed to the fact that such species usually produce heavy seeds with relatively limited means of long-distance dispersal. Macrofossils therefore typically represent only local vegetation changes, and macrofossil assemblages from different parts of the same lake may differ (Birks 2001). These patterns were echoed in macrofossil distribution studies in the UK (Zhao *et al.* 2006) and Turkey (Levi *et al.* 2014). Despite this, macrofossil reconstructions based upon a single sediment core are common, and rely upon the assumption that spatial distribution of macrofossils is even across a lake basin (Birks 1995; Birks 2001). Strategies for choosing core location are often based upon a) proximity to the littoral zone; b) ease of sampling and access; and c) known/assumed areas of sediment accumulation, rather than present day plant distribution (Birks 2001).

1.6 Combining ecology and paleoecology

The combination of documentary evidence, ongoing monitoring data and applied paleoecology has proved to be a powerful tool in assessing the causes and timing of plant community changes in lake ecosystems. Documentary records, often incomplete and only covering a short time scale, tend to reliably include most or all species that were present during the survey. Paleoecological records do not reliably represent all species (a study of Groby Pool, England, for example, showed that macrofossil records represented 40% of all species present in documentary evidence from the same site (Davidson *et al.*, 2005)), but do give a continuous record of ecological changes, potentially over a period of thousands of years. Davidson *et al.* (2005) were able to use the documentary evidence from Groby Pool to confirm a shift from *Chara* spp. dominance to *Potamogeton* spp. dominance recorded in the macrofossil record. In a separate study, Salgado *et al.* (2010) were able to use macrofossils found in a sediment core to establish that the plant community of Loch Leven, Scotland, had undergone a shift from *Isoetes* spp. dominance towards a *Potamogeton* spp. and *Chara* spp. community long before regular systematic monitoring of plants began. Macrofossil records have also been used to demonstrate that UK lakes from a variety of different geological settings, altitudes, depths, lake areas, trophic status, and current macrophyte flora have all experienced a loss of plant diversity over the past 150-200 years (Bennion *et al.*, 2017). To date, studies combining documentary records with paleoecology have focussed on the identification of whole-community changes, largely because such reconstructions aid identification of the timing and extent of anthropogenic pressures on lake systems. Given the uncertainties surrounding existing data on rare species and the increasing demands for evidence-based policies, the potential applications of the combination of contemporary ecology and paleoecology to rare species conservation should not be ignored.

This study aims to combine contemporary ecology with paleoecology to provide an evidence-base for the conservation of a single rare macrophyte: *Najas flexilis.* Like most rare macrophytes in the UK, a large quantity of data has been collected on *N. flexilis* since the introduction of SCMs in 2000, but these data have remained largely unexplored. Data before 2000 are scarce, and the methodologies used to collect the data unreliable.

Whilst SCM data provides the opportunity to learn more about the environments in which *N. flexilis* currently thrives in the UK, paleoecology, in combination with documentary evidence, can be used to determine longer-term shifts in the species and associated ecological changes and to explore the drivers of the observed changes.

1.7 Description of *N. flexilis*

Najas flexilis (Willd.) Rostk. & Schmidt, common name "Slender Naiad", is a submerged rooted macrophyte of the *Najadaceae* (Figs. 1.1 and 1.2). The *Najadaceae* family contains only one genus - *Najas* - of which there are 40 species. Most of these species are found in the tropics and subtropics, in fresh or brackish waters (Cook, 1996). There are two subgenera; *Caulinia* and *Najas*. *N. flexilis* belongs to *Caulina* (Triest, 1988). It is an elodeid, but, unlike many plants that live their entire life cycle underwater, is a relatively short plant, growing to a maximum height of ~30cm. It has linear, sessile, denticulate leaves, arranged in either an opposite or, more commonly, a whorled fashion (Stace, 2010). It is a monoecious annual and, unusually for an aquatic plant, cannot reproduce vegetatively (Hutchinson, 1975). The flowers are small and inconspicuous, with the male flowers at upper nodes and female flowers below (Preston and Croft, 1997). Fruits are sessile droops, and seeds are elliptical, long, and have a persistent membranous pericarp (Wingfield *et al.*, 2004).

Figure 1.1 Najas flexilis *(Willd.) Rostk. & Schmidt. A, flowering and fruiting stems; B, very young male flower held within the sheath; C, male flower at dehiscence; D, female flower; E, fruit; F, apex and lower part of a leaf. Source: Ross-Craig (1973).*

Figure 1.2 Photographs of N. flexilis. *Left: Growing in situ in Loch Ballyhaugh (photograph by author); top right: Leaf tip (photograph used with permission of Chris Carter); bottom right: Sheathed flowers at stem nodes (Chris Carter).*

1.8 Global distribution and decline

N. flexilis has a global circum-polar distribution (Fig. 1.3), but is found much more frequently in North America than in Europe (Godwin, 1975; Haynes, 1979). Its range in Asia is unknown, although it has been cited in Mongolia (Hultén and Fries, 1986). Within Europe, the climatic region of *N. flexilis* is defined as Boreal-montane, and the species is confined to altitudes <300 m (Preston and Hill, 1997a). The plant is considered native in the geographical range extending from the UK in the West to Russia in the East, and from Scandinavia in the north to a southerly limit in Switzerland. The largest European populations of *N. flexilis* are in Sweden, where there are thought to be around 10,500 individuals, and in the British Isles, where records exist from 51 sites in Scotland and 28 in Ireland according to the most recent study (Wingfield *et al.*, 2004; Gärdenfors, 2010).

Within the rest of its European range, *N. flexilis* has a very low frequency of occurrence – it is known from one locality in Denmark, five in Finland, six in Latvia and three in Lithuania (Stolze and Phil, 1998; Rassi *et al.*, 2001; Commission of the European Communities 2009). In recent decades, the European distribution of *N. flexilis* has been in decline, particularly in the south; the plant has not been seen in Germany since 1973, has declined in the Russian regions of Pskov, Novgorod, Tver and Moscow, and is thought to be extinct in Poland and Switzerland (Tzvelev, 2000; Kalinka and Nowak, 2004; Commission of the European Communities 2009). Because of these declines, *N. flexilis* is protected internationally as a 'Priority Species' within Annexes II and IV of the European Habitats Directive (1992). In the British Isles, *N. flexilis* has been found mainly on the Atlantic coastlines of both Scotland and Ireland, with clusters of sites found in Donegal, Connemarra and Kerry in Ireland and the Western Isles, Inner Hebrides and Perthshire in Scotland (Fig. 1.4). Due to the important contribution that the UK makes to the European distribution of *N. flexilis,* the plant is protected under domestic legislation in additional to its international designations. It is listed in Schedule 8 of the Wildlife and Countryside Act (1981) and as a priority species in the UK Biodiversity Action Plan (2008).

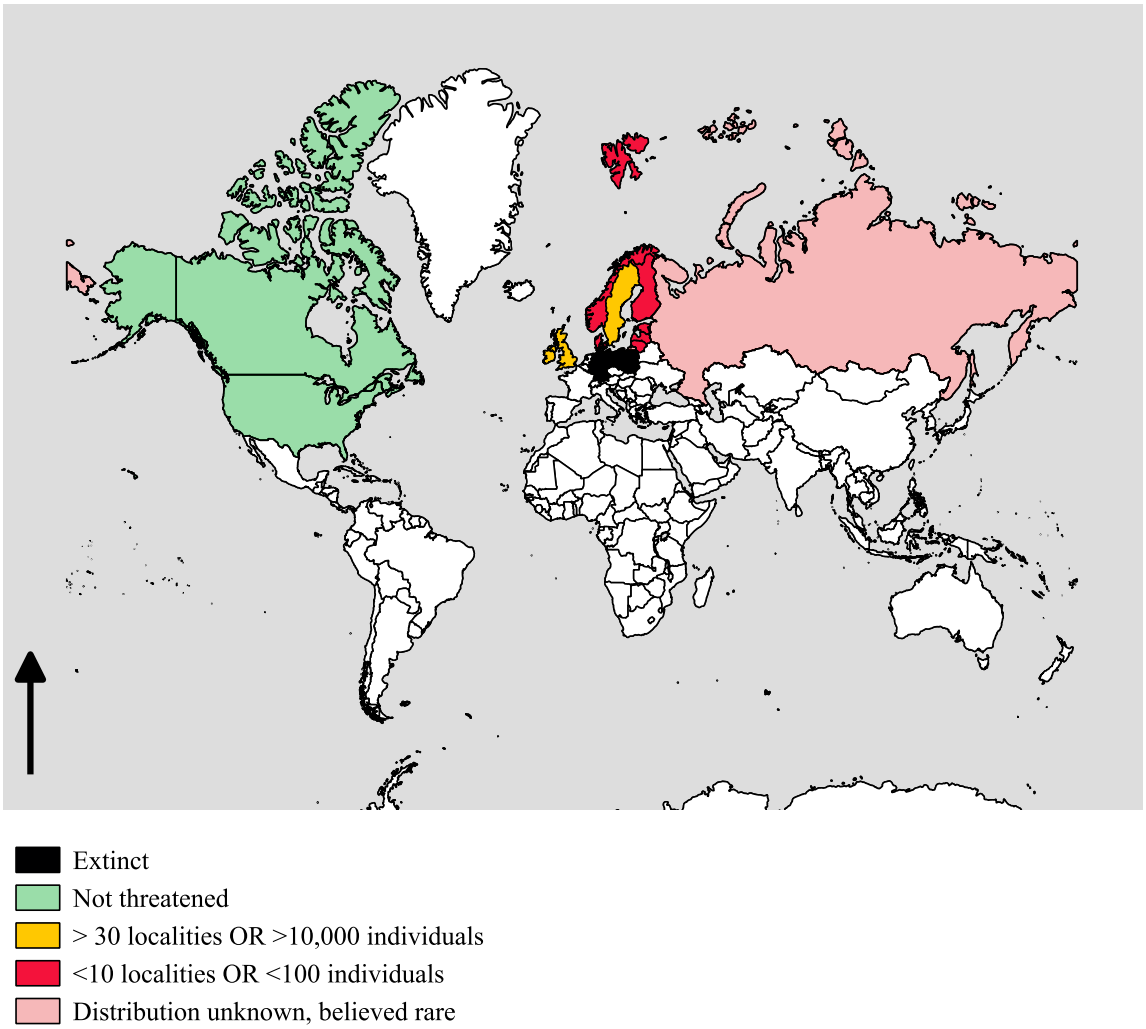

Figure 1.3 Current global distribution of N. flexilis*. Data from Commission of the European Communities (2009).*

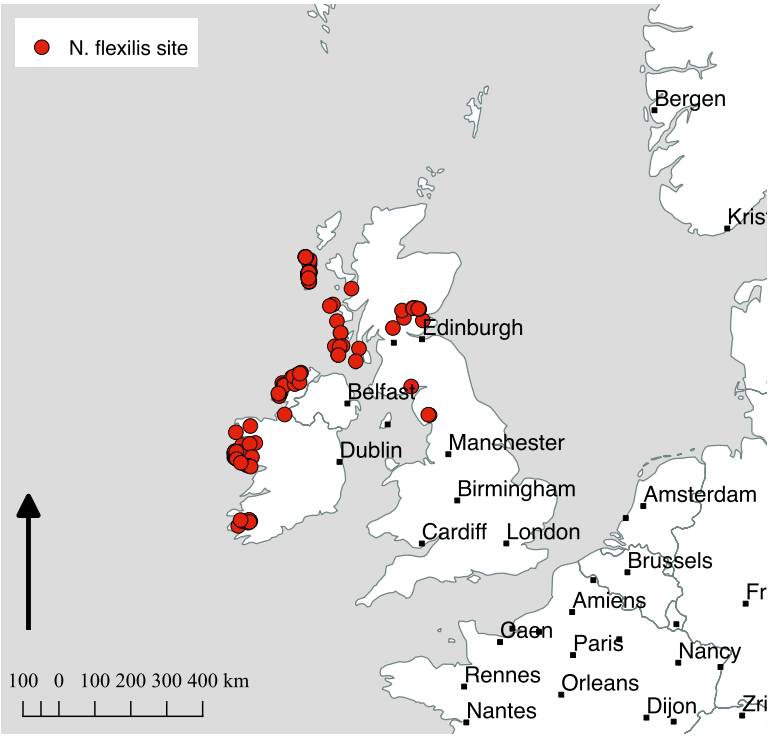

Figure 1.4 Sites at which N. flexilis *has been recorded in the UK, 1850 - present.*

As a protected species, *N. flexilis* is subject to regular monitoring as part of the SCM program. However, although historical records on *N. flexilis* occurrence in the UK are available from a variety of sources, the long-term dataset contains numerous large gaps during which the presence/absence of the species at many sites is unknown. *N. flexilis* was first recorded in Cregduff Lough, Ireland, in the mid – nineteenth century (Oliver, 1851). The first Scottish record is from Loch Clunie in 1875 (Sturrock, 1875). Since that time, monitoring of *N. flexilis* has been far from consistent, and surveys exist from four main periods. The first, in the 1920s and early 1930s, aligns with the interest of eminent botanists such as Pearsall (e.g. Pearsall, 1915). The second reflects a period of increased interest following the disappearance of the plant from England in the 1980s (e.g. James and Barclay, 1996). The third represents the PhD research of Wingfield (2002), who undertook a large-scale survey of *N. flexilis* sites between 1998 and 2000. Finally, the introduction of SCM in 2000 has led to an increase in the number of sites surveyed for *N. flexilis* in the 21st century. Despite this, the only database of *N. flexilis* records in the UK,

held by SNH, is both out-of-date and incomplete, and the extent to which changing British populations of *N. flexilis* echo the declines observed elsewhere in Europe is unknown.

1.9 Inconsistencies in Monitoring

Not only are existing records sparse, but the methodologies used in ongoing SCMs are inconsistent. The Site Condition Monitoring (SCM) Guidance for freshwater lakes outlines the current methodologies recommended for the regular monitoring surveys of plant communities and rare plant species in SACs and SSSIs (Gunn *et al.*, 2010). The guidance for habitat and community surveys is extremely detailed, and designed to collect as much information about each lake as possible with realistic limitations on time and money spent on each survey. Where protected species are present, additional monitoring is required as part of the SCM to ensure that the species is adequately captured by the survey; however, this guidance does not contain the same level of detail as is given for standard habitat surveys. It is suggested that two distinct 'populations' of the target species are identified and recorded at each site, and the presence/absence of the species is recorded in 20 random quadrats within each 'population' (Interagency Freshwater Group, 2015). In the case of plants that occupy the deeper water, snorkel and/or SCUBA surveys been recommended as an alternative strategy that allows direct observation of the lakebed (Gunn *et al.*, 2004). Aside from this, there is little guidance given on how to conduct these surveys, and survey methodologies and sampling strategies vary in time and space as a result.

In a study of Whalebone Cove, USA, Capers (2000) showed that *Zannichellia palustris, N. flexilis* and *Najas minor,* were all susceptible to being under-recorded using a grapnel; *N. flexilis* was not recorded at all by a boat-based grapnel survey despite being present in 7.7% of the same quadrats when observed by snorkel. Although snorkel surveys for *N. flexilis* are recommended for SCM, they are perceived to be expensive, require specific equipment and expertise, and to carry inherent risks that require special health and safety

procedures. This means that there is some resistance to adopting this approach even where it is strongly recommended, and grapnels are more commonly used (Stewart, pers. comms). Compared with bathyscopes, Ekmann grabs and underwater photography, grapnels have been shown to give the most accurate representation of macrophyte communities in terms of both number of species recorded and the maximum colonisation depth (Spears *et al.*, 2009). However, they are thought to under-represent some species, particularly those that are very small and/or slender. In the UK environment, where many lakes are much smaller than those studied in the USA, few studies have directly compared the effectiveness of grapnel and snorkel/SCUBA surveys, which further contributes to the reluctance of some to adopt the snorkel approach, compounding the inconsistency in survey methods for *N. flexilis* between surveys. There is clearly a need to establish an evidence base to support decision making when designing future *N. flexilis* surveys.

The SCM methods are designed to cover as much of the habitat diversity present at any one site as possible. However, focussing on shoreline sectors and transects that extend to maximum wader depth introduces a sampling bias against deeper waters. This is exacerbated by the fact that it is not always possible to deploy a boat for the boat transects, for example in high winds or where dense plant beds are present. As a deep-water aquatic, *N. flexilis* is particularly susceptible to under-recording in SCM. There is provision in the SCM for extra survey effort for rare species; two separate populations of the target species must be identified, and 20 1m2 quadrats must be sampled within each population (Interagency Freshwater Group, 2015). Unlike the standard SCM transects, there is no requirement to assess the same populations in the same locations in subsequent years. This maximises the chances of finding the target species if it is present, but means that there is no temporal consistency in the sampling strategy and populations surveyed in one year cannot be directly compared to those surveyed in another year. Furthermore, *N. flexilis* populations within a lake can be difficult to locate even when the locations of previous populations are known. Without a consistent approach to the monitoring of *N. flexilis* populations in the British Isles, it is difficult to collect an

evidence-base for ongoing conservation of the species. Chapter 3 aims to address this problem by creating a standardised monitoring protocol for future SCMs of *N. flexilis.*

1.10 Ecology of *N. flexilis* and potential causes of decline

In the absence of both consistent monitoring techniques and focussed analyses of the SCM datasets that do exist, much of what is known about the ecological preferences and potential threats to *N. flexilis* comes from studies from North America. Here, *N. flexilis* prefers sites with a circumneutral pH and alkalinities between 6 mg l^{-1} and 308mg l^{-1} (Jackson and Charles, 1998; Moyle, 1945; Roberts *et al.* 1985). pH has been shown to be an important variable to the reproductive cycle of *N. flexilis;* Titus and Hoover (1991; 1993) found that seed production per *N. flexilis* plant dropped from an average 95.5 seeds per plant to 0.25 seeds per plant when water pH was reduced from 7.5 to 5. This effect was demonstrated outside of the laboratory following the liming of Thrush Lake, Minnesota, which led to an increase in the population of *N. flexilis* within the lake during the period of elevated pH (Hagley *et al.*, 1996). Alkalinity is important for the growth of the plant itself – specifically, calcium and magnesium levels. Wetzel and McGregor (1968) demonstrated that, whilst calcium levels up to 10 mg l^{-1} and magnesium levels up to 5 mg l^{-1} appear to increase carbon fixation, levels higher than this lead to a big decline in carbon fixation in *N. flexilis* plants. This means that *N. flexilis* prefers soft water lakes, and, as such, acidification is a commonly cited threat to the species (Wingfield *et al.*, 2004; 2006).

Between 1998 and 2000, Wingfield *et al.* (2004) carried out an extensive investigation into the ecology of *N. flexilis* in the UK and Ireland. They compared water and sediment chemistry between two groups of lakes; 42 at which *N. flexilis* was present and 9 of which formerly contained populations of *N. flexilis* which have since disappeared. The variables that most strongly correlated with *N. flexilis* presence were concentrations of magnesium, calcium, phosphate, alkalinity, pH and sediment phosphate concentration. Based upon these findings, Wingfield (2002) postulated that acidification is a likely threat to *N. flexilis* in the UK. The majority of lochs in which *N. flexilis* is found are

lowland, mesotrophic lakes that lie on a base rich geology (Wingfield *et al.*, 2006). Many of the sites in western Scotland, for example, have a high calcium concentrations and alkalinities deriving from the Machair sands of the Western Isles on which they lie (Waterston *et al.*, 1979). The base-rich geology of these lakes does not put them at a high risk from acidification, although there are some sites in a more inland location that may be susceptible to this threat.

An alternative hypothesis for the UK decline of *N. flexilis* is eutrophication. In their study, Wingfield *et al.* (2004) showed that phosphate concentrations in both the water and the lake sediments were correlated with *N. flexilis* presence/absence. Wingfield *et al.* (2005) showed that many lakes from which *N. flexilis* had recently disappeared were associated with species compositions typical of the most nutrient-rich lakes included in the study. Three of the lakes in the UK from which *N. flexilis* is believed to have disappeared – Esthwaite Water in Cumbria, and Loch Marlee and Loch of Butterstone in Perthshire – are known to have been subject to nutrient enrichment. However, several studies have shown that light is not a limiting factor for *N. flexilis* growth, and *N. flexilis* often grows in deep areas of a lake alongside other species that tolerate low light levels, such as *Nitella* spp. (Pearsall, 1920; Hough and Fornwall, 1988; Wingfield, 2002). The mechanism by which eutrophication subdues *N. flexilis* growth is therefore not fully understood.

Another potential threat to *N. flexilis* in the UK is competition from the invasive species *Elodea canadensis* and *Elodea nuttallii*. *Elodea canadensis* is native to the Americas and was introduced to Ireland in 1836, whilst the more aggressive *Elodea nuttallii* was first recorded in a ditch in Oxfordshire in 1966 (Simpson, 1984). In both the USA and the UK, *Elodea* spp. are known to live in similar conditions and at similar water depths to *N. flexilis* (Sheldon and Boylen, 1975; Wingfield *et al.*, 2005). In the UK, detrimental impacts on *N. flexilis* growth because of *Elodea* spp. invasion have been reported only from Tangy Loch (Argyll); at most sites where both species are present, they are believed to coexist (Wingfield *et al.*, 2004). Both *Elodea canadensis* and *Elodea nutallii* have recently been introduced to the Western Isles (Wingfield *et al.*, 2004) and it is difficult to predict the likely impacts on *N. flexilis* populations based on current knowledge on the

interactions between the two species.

Although Wingfield *et al.* (2004) made significant progress in determining the ecological factors that influence *N. flexilis* growth in the UK, several questions remain unanswered. Significant overlap in the ranges of water chemistry variables was found between lakes in which *N. flexilis* was present and those in which it was absent, and no differentiation was made between sites at which *N. flexilis* was abundant and those at which it was rare. Often, *N. flexilis* was only locally abundant within a lake, and the factors that determined the zone of *N. flexilis* growth were unclear – neither water depth nor light extinction coefficient, for example, were correlated with *N. flexilis* presence/absence. It has also been casually observed that *N. flexilis* tends to be associated with fluid sediments, although this has not been formally measured (Wingfield *et al.*, 2004). However, another *Najadiceae* - *Najas marina* - has been observed to be closely associated with a thick, fluid sediment at Upton Great Broad, Norfolk (Ayres *et al.*, 2008). At Thrush Lake, Minnesota, *N. flexilis* thrived in holes that were created by anchors and nets, and it is therefore possible that *N. flexilis* has a competitive advantage in areas of sediment disturbance (Hagley *et al.*, 1996). Furthermore, *N. flexilis* is not associated with a single plant community, but grows in association with different macrophytes in different situations (Wingfield *et al.*, 2005). The factors that define the micro-habitat in which *N. flexilis* grows are subject to change both in conjunction with, and independently from, acidification, eutrophication and *Elodea* spp. invasions.

Using TWINSPAN analysis of 52 current and former *N. flexilis* sites in Scotland and Ireland, Wingfield *et al.* (2005) showed that the plant thrives alongside different plant communities under different water chemistry conditions, ranging from more oligotrophic/acidic *Isoetid* communities to more mesotrophic/alkaline *Potamogeton* communities. This study was based on water chemistry and whole-site species composition data collected on a single visit to each lake, and did not include any information about the micro-habitat occupied my *N. flexilis* (e.g. substrate type, water depth and disturbance). Since this study was published, data from three SCM cycles, spanning a period of sixteen years, have been collected from many *N. flexilis* sites in

Scotland. These data include the species composition, water depth and substrate type of each individual sample point within each lake, and the most recent SCM cycle made use of the survey protocol described in chapter 3. Chapter 4 of this study aims to use this SCM data to further investigate the types of environments in which *N. flexilis* grows, with an emphasis on identifying the causes of recent changes in *N. flexilis* distribution in Scotland.

1.11 Paleoecological records of *N. flexilis* change

Although SCM data can be used to investigate the current status of *N. flexilis,* the absence of extensive long-term monitoring records makes paleoecology the only viable way to determine changes in *N. flexilis* populations that occurred pre-2000. On a Holocene timescale, sub-fossil seed fragments of *N. flexilis* are abundant in early sediments from the UK. This suggests that the plant was more widely distributed during the post-glacial climatic optimum (circa 7500 – 5000 B.P.) than currently (Pennington, 1974). Records dating from this time have been found at Hockham in Norfolk (Godwin and Tallintire, 1951), Nazeing in Essex (Allison *et al.*, 1952), Tregaron Bog in Wales (Godwin and Mitchell, 1938) and Kentmere in the Lake District (Walker, 1955). It has been suggested that the decline in *N. flexilis* distribution after this period was mainly due to the thermophilous nature of the plant and decreasing temperature during the latter part of the Holocene (Pennington, 1974). However, it is clear from the botanical records that the plant has persisted in locations where other ecological conditions remained suitable across northern England, Scotland and Ireland. Paleoecological studies covering the more recent past suggest that *N. flexilis* abundance may be affected by anthropogenic influences on lake habitats. In Poland, declines and disappearances of *N. flexilis* seeds in cores from two lowland forest lakes appear to correlate with changes in the trophic status, with *N. flexilis* decline correlating with an increase in *Sphagnum* spp. remains in a core from the Rominicka Forest (attributed to nutrient depletion/acidification) and an increase in *Botryococcus* spp. in a core from Lake Linówek (attributed to eutrophication) (Gałka *et al.*, 2012). In contrast, the paleolimnological work of Bennion *et al.* (2008; 2010), which included seven lakes in Scotland currently or formerly supporting *N. flexilis*, did

not support the theory that *N. flexilis* is in decline. Instead, it appeared that some sites may have been too unproductive for *N. flexilis* in the past, with macrofossil remains of the species appearing alongside diatom and cladocera assemblages indicative of mild eutrophication. At Castle Lough, Ireland, macrofossils suggested that *N. flexilis* had declined in one of three basins of varying depth in response to lake-wide eutrophication, but had maintained populations of low abundance in both the shallowest and the deepest basin (Salgado *et al.*, 2017). However, all of these studies are based upon analysis of cores at a low resolution, and steady trends of change in *N. flexilis* seed numbers throughout the cores were not observed. Furthermore, the seed distribution patterns of *N. flexilis* were neither fully investigated nor taken into account when choosing core locations or interpreting results.

1.12 Suitability of *N. flexilis* for paleoecological study

In order to understand and interpret macrofossil records, the relationship between sediment seed distribution and the distribution of the plant with a lake must be understood. A study by Zhao *et al.* (2006) compared the spatial distribution of macrofossils in the surface sediments of Green Plantation Pond, a small, shallow lake in England, with the spatial distribution of the macrophytes themselves. They demonstrated that different plants produce different numbers of remains and distributed them across the lake in different ways – *Zannichelia palustris*, for example, was significantly over-represented in the sediment record whilst *Potamogeton* spp. were significantly under-represented. A similar study spanning 35 shallow lakes in Turkey found similar relationships, with *Chara* spp. also significantly over-represented in the sediment record across all sites (Levi *et al.*, 2014). Ideally, in order to ensure the capture of the target species and to prevent over- or under-representation, multiple cores should be collected from the same site and cross-correlated using repeatable stratigraphic features. However, this can be time-consuming, expensive and prone to error (Patmore *et al.*, 2014). In Minnesota, Birks (1973) noted three exceptions to the general, localised representation of obligate aquatic plants as seed macrofossils; *Chara* spp, *Nitella opaca* agg., and *N. flexilis.* Such a distribution pattern potentially eliminates the need for multiple cores,

because remains are likely to be found in cores taken from any location within a basin containing the plant. The oospores of the *Characeae* are small and easily distributed by water currents, and this has been observed in surface sediments and in sediment cores across the world (e.g. Zhao *et al.* 2006; Madgwick *et al.* 2011; Levi *et al.* 2014). However, no such dispersal mechanism is currently known of in *N. flexilis,* which produces relatively large, heavy seeds. In order to better understand the seed dispersal patterns of *N. flexilis* to the extent that it is relevant to paleoecology, chapter 5 investigates the relationship between parent plants and seeds in the sediments.

Provided that *N. flexilis* seed distribution patterns can be understood, the species is well suited for paleoecological study. *N. flexilis* has distinctive elliptical seeds with a dark, shiny pericarp, which are easily identified and preserved in lake sediments (Fig. 1.5). Furthermore, many of the perceived threats to *N. flexilis* are readily explored through paleoecological proxies. Plant macrofossils are widely used to reconstruct changes in the submerged vegetation of lakes that commonly results from eutrophication (e.g. McGowan *et al.*, 2005, Salgado *et al.*, 2010, Bennion *et al.*, 2017), and have also been shown to reflect trophic cascades following changes in fish predation (Sayer *et al.*, 2016). Diatoms are most commonly used to demonstrate acid deposition (Battarbee *et al.*, 2010), but can also be applied to changes in lake trophic status (Hall and Smol, 2010). Cladocera are positioned in the middle of the food web, so are particularly sensitive to a variety of ecosystem changes (Davidson *et al.*, 2011; Tolotti *et al.*, 2016). The sub-fossil remains of Cladocera can therefore be used in investigations of lake level changes (Nevelainen *et al.*, 2011), climate change (Korponai *et al.*, 2011; Zawiska *et al.* 2015), salinity fluctuations (Amsinck *et al.*, 2005), trophic shifts (Hofmann, 1996), macrophyte abundance (Thoms *et al.*, 1999, Johansson *et al.*, 2005 read both), and fish predation (Leavitt *et al.*, 1989). Within the United Kingdom, *N. flexilis* is known to have been lost from Esthwaite Water in England in the 1980s, and is believed to have disappeared from the Dunkeld-Blairgowrie chain of lochs in Perthshire, Scotland, in the 2000s (Wingfield *et al.*, 2004). Both Esthwaite Water and the Dunkeld-Blairgowrie lochs were designated as Special Sites of Scientific Interest (SSSIs) and, in the case of the latter, a Special Area of Conservation (SAC), in part because of their populations of *N. flexilis*. They have also

been subject to a variety of anthropogenic pressure, including eutrophication, aquaculture, and invasive species introduction (Bennion *et al.*, 2010; Vernon and Hamilton, 2011; Dong *et al.*, 2011; Dong *et al.*, 2012). Chapter 6 uses multi-proxy paleoecological techniques to explore long-term changes in *N. flexilis* populations, and associated shifts in other biological groups, at these sites.

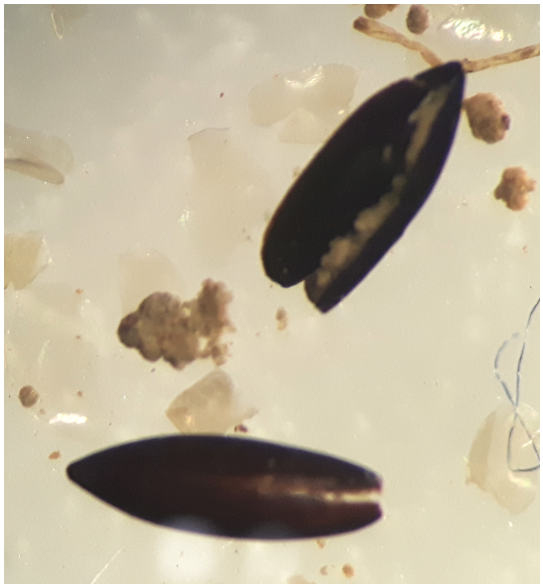

Figure 1.5 N. flexilis *seeds found within a sediment core (photograph by author). Seeds measure ~2mm in length.*

1.13 Research aims and objectives

The aim of this PhD is to use contemporary ecology in combination with paleoecology to provide an evidence-base for the conservation of *N. flexilis* in the UK. The key objectives are:

1. To establish an improved protocol for monitoring inter-annual changes in *N. flexilis* populations, that can be used alongside current SCM techniques.
2. To use existing SCM data alongside new data to investigate the types of environments that *N. flexilis* grows in, with an emphasis on identifying the causes of recent changes in *N. flexilis* distribution in Scotland.

3. To explore the ways in which knowledge of seed dispersal patterns might inform paleoecological macrofossil reconstructions of *N. flexilis*.
4. To conduct paleoecological investigations into the decline of *N. flexilis* at Esthwaite Water and Loch of Craiglush (the first loch in the Dunkeld-Blairgowrie chain).

1.14 Thesis outline

The research design, study sites and methodologies used in this PhD are described in chapter 2. Each of the four research objectives is then discussed in the order presented above, which each of chapters 3 – 6 devoted to one of the objectives. Chapter 7 more broadly discusses the lessons that can be drawn from this research and how it may be applied to the practical conservation of *N. flexilis* in the future, as well as outlining areas for further research.

2. Methodologies and Site Descriptions

2.1 Research Design

In order to select sites for study, a database of all existing *N. flexilis* records was created. After systematically contacting all of the people in the UK who were recognized as having worked with *N.* flexilis and/or rare macrophytes, seven main sources of historical data on *N. flexilis* presence/absence were identified: the National Biodiversity Network (NBN Gateway, 2012; NBN, 2014), Scottish Natural Heritage (SNH) (Hennessey, unpublished), Natural History Museum herbarium (NHM) (unpublished), Chris Preston (pers. comms, unpublished), Wingfield *et al.* (2004), Kindrogan water plants course data (Stewart, unpublished) and the Irish Environmental Protection Agency (O'Conner and Roden, pers. comms, unpublished). The data were collated into a single, searchable database along with any other incidental findings from the literature, and used to compile a complete list of sites in the British Isles in which *N. flexilis* has been found. The database is summarised in appendix 1.

Sites in Ireland were excluded from this study on the basis that a separate study focussing on *N. flexilis* in Ireland was already underway (O'Conner and Roden, pers. comms.). From the Scottish sites, three sites were selected for pilot studies to test different sampling methodologies and to investigate the relationship between *N. flexilis* plants and seeds in the surface sediments. These three sites were Upper Glenastle Loch (latitude 55.621389; longitude -6.290278), Tangy Loch (latitude 55.492213; longitude -5.652543) and Loch of Butterstone (latitude 56.585862; longitude -3.533379). They were chosen because they were located on the Scottish mainland or the Inner Hebrides and therefore were relatively easy to access, and because, together, they represent lakes with *N. flexilis* distributions from highly abundant (Upper Glenastle Loch) and moderately abundant (Tangy Loch) to recently extinct (Loch of Butterstone). Following the pilot study, the new survey protocol was honed in 2016 during SCM surveys of sites that currently or formerly contained *N. flexilis* in Scotland. Between July and September 2016, 21 of the 26 Scottish *N. flexilis* sites that are subject to SCM were visited. The contemporary

distribution of *N. flexilis* in Scotland was studied using data from the 2016 surveys, the three pilot studies, and past monitoring data from all 26 Scottish *N. flexilis* sites that are subject to SCM. Paleoecological studies focussed on the two sites from which the disappearance of *N. flexilis* has been well documented: Esthwaite Water, England, and the Dunkeld-Blairgowrie Lochs, Perthshire, Scotland. The way in which each stage of the research relates to the specific study objectives is summarised in Fig. 2.1.

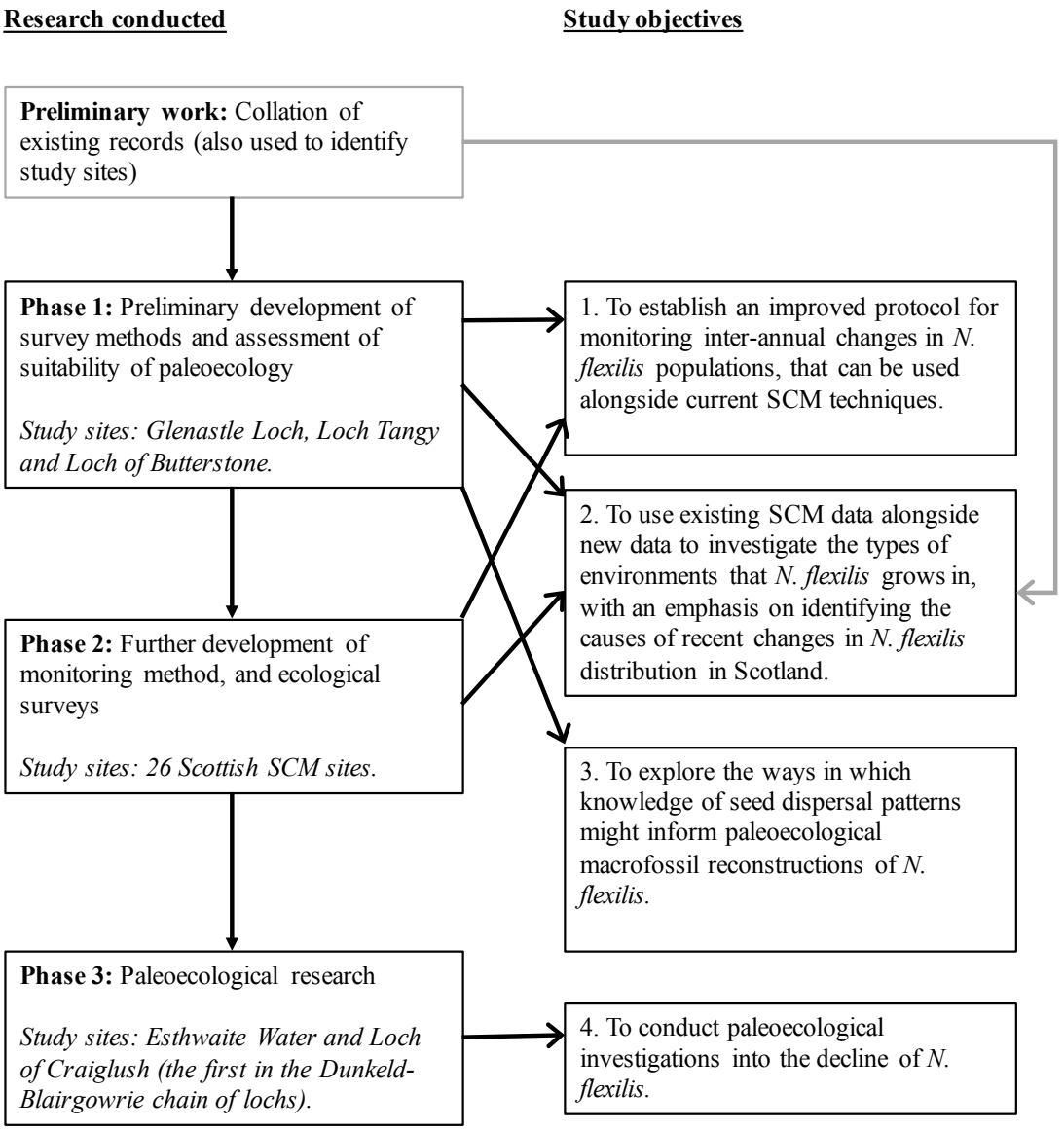

Figure 2.1 Research design, with relation to specific study objectives

2.2 Study sites

2.2.1 Phase 1: Preliminary development of survey methods and assessment of suitability of paleoecology

a) Glenastle Loch (upper basin, hereafter referred to as "Glenastle Loch")

Glenastle Loch (latitude 55.621389; longitude -6.290278, Fig. 2.2) is the first in a chain of two lochs on the 4km long Glen Golach, a river located on the Oa peninsula on the island of Islay in the Inner Hebrides. The loch is 8 ha in area, with a small catchment of 272 ha. It is a shallow (4.9m mean depth) lake with a westerly prevailing wind (RenSmart 2016). It is classified as a "moderate alkalinity/mesotrophic" lake for the purposes of UKBAP and the Water Framework Directive, and falls into the "oligotrophic to mesotrophic" category of the EU Habitats Directive (Hughes *et al.*, 2004). The catchment lies on the Glen Egedale Slate formation, with overlying peat deposits in the surrounding Maol Mhòr uplands. 60% of the catchment is open dwarf shrub heath, 30% is unimproved acid or neutral grassland, and the remaining 10% is a mix of woodland and bare ground (Fuller *et al.*, 2002). The entire Oa peninsula, including Loch Glenastle, is an RSPB nature reserve. It is protected predominantly for its population of rare seabirds (chough and golden eagle), although the freshwater habitats also attract hen harrier and corncrake (RSPB, 2017). Past surveys show that *N. flexilis* was present in Upper Glenastle Loch in 1994, 1998, 1999 and 2010 (National Biodiversity Network, 2014). None of these records indicate the extent or distribution of *N. flexilis* cover, however it is believed to be relatively abundant at the site (Hennessey, pers. comms.)

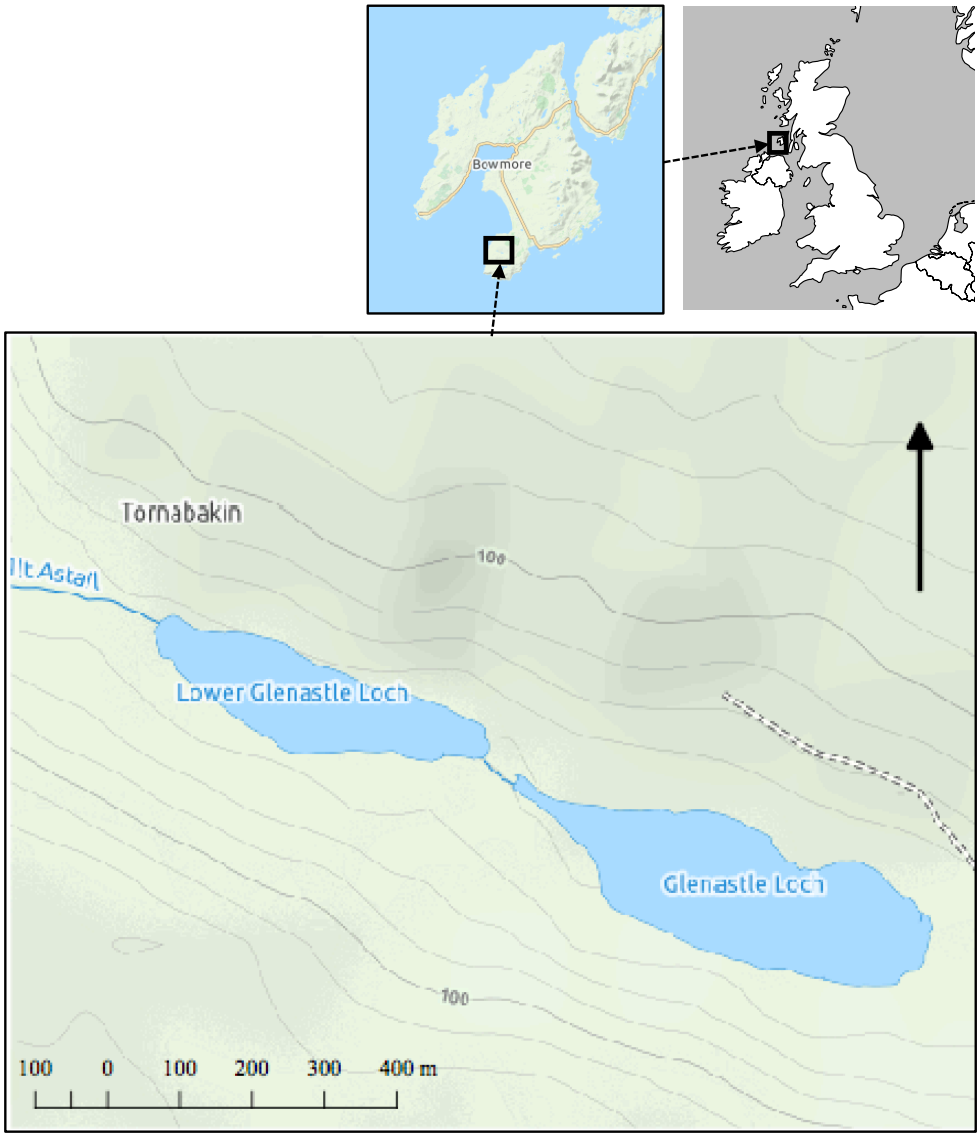

Figure 2.2 Map of Glenastle Loch, Islay, Inner Hebrides, Scotland.

b) Loch Tangy

Tangy Loch (latitude 55.492213; longitude -5.652543, Fig. 2.3) is located on the Kintyre peninsula, Argyll and Bute, mainland Scotland. It is a small (19 ha), shallow (mean depth 5 m) lake with a 175 ha catchment and a prevailing wind from west-south-west (RenSmart, 2016). It is classified as "moderate alkalinity/mesotrophic" in UK legislature, and "oligotrophic to mesotrophic" in the EU Habitats Directive (Hughes *et al.*, 2004). The lake and catchment lie on the Glen Sluan Schist formation, with an overlay of

Devensian till. 70% of the catchment is used for coniferous forestry, with the remaining 30% comprised of neutral/acid grassland and open dwarf shrub heath (Fuller *et al.*, 2002). Past surveys found *N. flexilis* in Tangy loch in 1973, 1977, 1978, 1983, 1989, 1994, 1998 and 2009 and 2014 (National Biodiversity Network, 2014). The 2014 survey indicated a small but healthy population of the plant (Scottish Natural Heritage, pers. commun., Walmisley, pers. commun.).

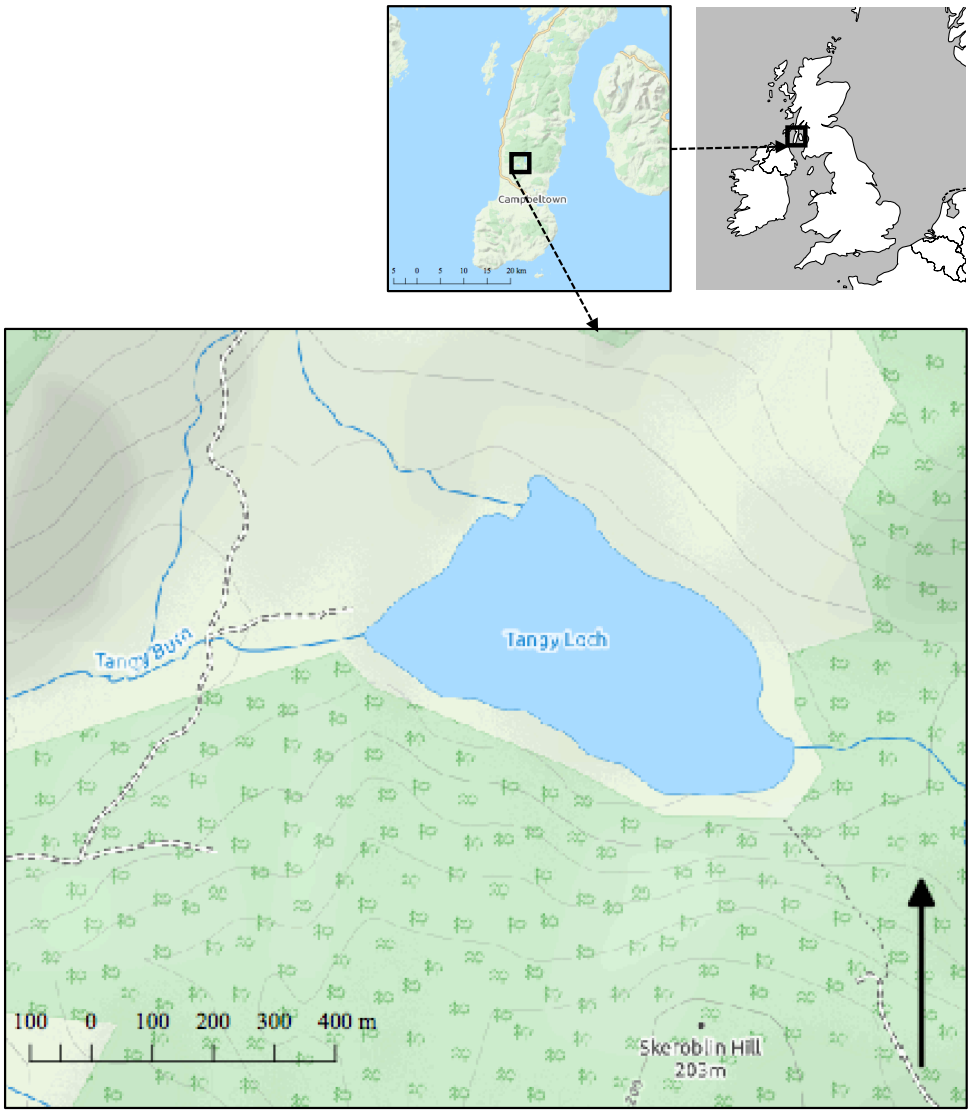

Figure 2.3 Map of Tangy Loch Kintyre peninsula, mainland Scotland.

c) Loch of Butterstone

Loch of Butterstone (latitude 56.585862; longitude -3.533379) is the third loch in a chain of five lochs known as the "Dunkeld-Blairgowrie Lochs", located along the Lunan Burn river in Perthshire, mainland Scotland. These lochs are described in more detail on page 50 and the upper three lochs, including Loch of Butterstone, are shown in Fig. 2.4 (overleaf). The mean depth of Loch of Butterstone is 3.4 m. It is 43 ha in area, and has a prevailing wind from west-south-west (RenSmart, 2016). It is classified as "moderate alkalinity/mesotrophic" in the UK, and "oligotrophic to mesotrophic" in the EU Habitats Directive (Hughes *et al.*, 2004). The catchment area is 2226 ha, and lies partly on Semipelites and Psammites and partly on Micaceous rocks of the Southern Highland Group formation (Walker, 1961). Catchment land use is mixed, comprising 24% mixed woodland, 22% coniferous plantations, 18% dense dwarf shrub heath, 13% improved pasture, 11% unimproved acid/neutral grassland, and smaller percentages mixed arable farming, inland waterbodies and bare ground (Fuller *et al.*, 2002). Past surveys show that *N. flexilis* was present in Loch of Butterstone in 1986, 1994, 1996, 1997, 1999 and 2004 (James and Barclay, 1998; Wingfield, 2006), but has not been observed since.

2.2.2 Phase 2: Further development of monitoring method, and ecological surveys

In addition to the above sites, 24 sites were included in the investigation of the current environments occupied by *N. flexilis* by virtue of the fact that they are a) subject to SCM and b) were known to currently or formerly contain *N. flexilis* (Loch of Butterstone was both a pilot study site and an SCM site, bringing the total number of Scottish *N. flexilis* SCM sites to 26). Of these 24 sites, 21 were visited in 2016 and surveyed directly, using methods comparable with previous SCM surveys (see section). Previous SCM data was available for 15. These sites are summarised in table 2.1.

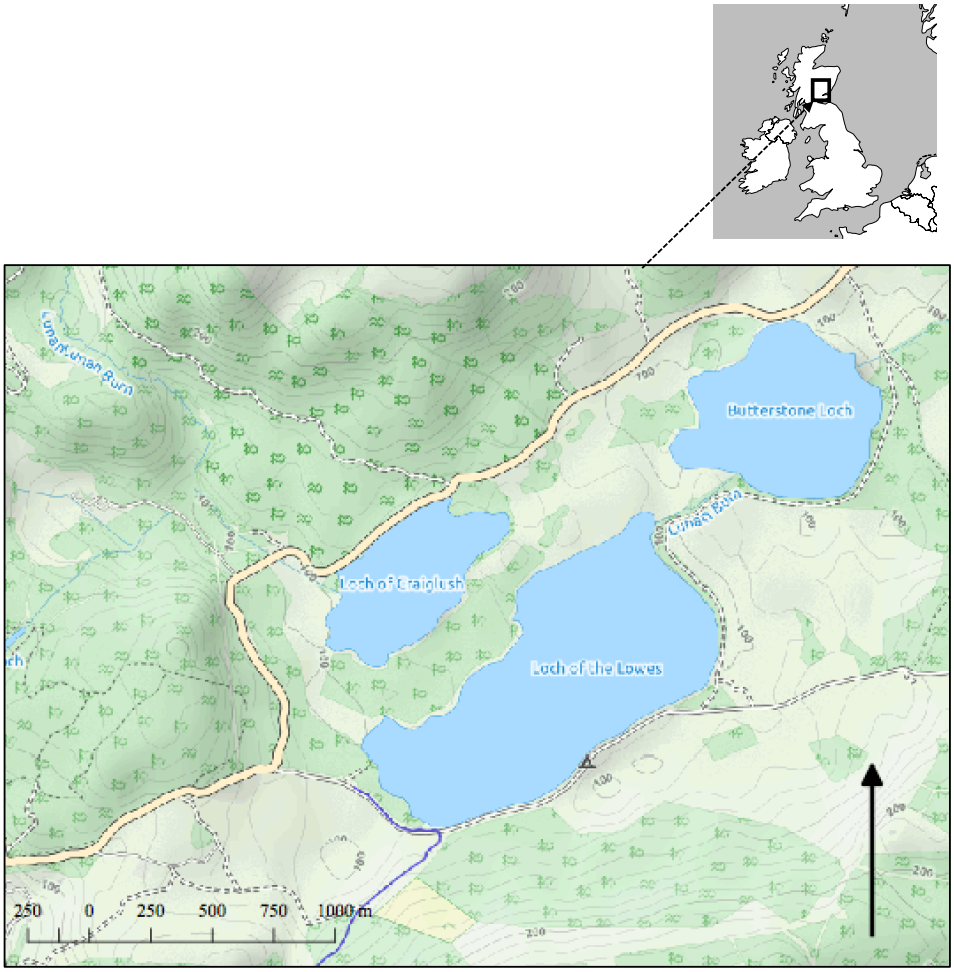

Figure 2.4 Map of Loch of Butterstone, Loch of Craiglush and Loch of Lowes (the upper three lochs in the Dunkeld-Blairgowrie chain, Perthshire, mainland Scotland).

Table 2.1 Sites included in ecological analyses, and definitions of site abbreviations.

Site	Site code	Latitude Longitude	Past *N. flexilis* records	Past SCM surveys	Visited in 2016?
Mainland Scotland					
Loch of Butterstone	But	56.585862 -3.533379	1986; 1994; 1996; 1997; 1999; 2004	2004; 2010	Yes
Loch of Craiglush	Cra	56.581024 -3.5587211	1967; 1977; 1994; 1995; 1996; 1997; 2000; 2004	N/A	Yes
Loch Clunie	Clu	56.580976 -3.4436147	1986; 1998; 2001	2004; 2010	Yes
Loch of Lowes	Low	56.577283 -3.5489623	1879; 1883; 1967; 1970; 1974; 1975; 1983; 1984; 1986; 1987; 1988; 1989; 1993; 1994; 1995; 1996; 1998; 1999; 2000; 2002; 2003; 2004; 2006; 2007	2010	Yes
Loch Marlee	Marl	56.582577 -3.3979257	1877; 1888; 1889; 1880; 1881; 1882; 1883; 1884; 1904; 1914; 1994; 1997; 1999; 2002; 2004; 2007	2010	Yes
Lake of Menteith	Men	56.174328 -4.292251	1994; 1995; 1997; 2000; 2002; 2004	2009	No
Tangy Loch	Tan	55.492213 -5.652543	1973; 1977; 1978; 1983; 1989; 1994; 1998; 2009; 2014	N/A	No
Inner Hebrides					
Loch Ballyhaugh	Ba	56.629143 -6.6059321	1987; 1989; 1994; 1999; 2004; 2009	2004; 2009	Yes

Loch Fada	Fad	56.080458 -6.2076994	1902; 1908; 1983; 1994; 1999; 2004	2004; 2009	No
Glenastle Loch	Gle	55.621389 -6.290278	1994; 1998; 1999; 2010	N/A	No
Loch an T'Sagairt	Tsa	56.659681 -6.4893360	1989; 1994; 1999; 2009	2004; 2009	No
Western Isles					
Loch Bun an Ligidh	Bun	57.309655 -7.3680906	1994; 2000	N/A	Yes
Loch Cuilc	Clc	57.308078 -7.3628715	1994; 1998	N/A	Yes
Loch Nam Cnamh	Cna	57.294850 -7.3775641	1994; 1999; 2010	N/A	Yes
Loch na Cuithe Moire	Cth	57.184935 -7.4013157	1983; 1994; 2000; 2004; 2010	2004; 2010	Yes
Loch Druidibeg	Dru	57.310241 -7.3332539	1987; 1994; 2000; 2004; 2010	2004; 2010	Yes
Loch an Eilean (Drimsdale)	Eil	57.310053 -7.3797897	1984; 1994; 1999; 2004; 2010	2004; 2010	Yes
Loch Gearraidh Mhic Iain	Ger	57.299392 -7.3765663	1994; 1999; 2004; 2009; 2010	N/A	Yes

Loch Grogary	Gro	57.613172 -7.5084828	1942; 1955; 1982; 1994; 1995; 1999; 2004; 2008; 2010	2004; 2010	Yes
Loch a' Mhadaidh	Mha	57.293061 -7.3773024	1994; 1999; 2010	N/A	Yes
Mid Loch Ollay	MLO	57.261661 -7.3959646	1987; 1994; 2000; 2004; 2010	2004; 2010	Yes
Loch ? (No name)	NoN	57.307654 -7.3727868	1994; 2000	N/A	Yes
Loch Phuirt Midh	Phu	57.187879 -7.3888743	n/a - *N. flexilis* found for the first time during 2016 SCM.	N/A	Yes
Loch Scaraidh	Sca	57.604668 -7.4971327	1994; 1995; 2000; 2010	2010	Yes
Schoolhouse Loch	Sco	57.302970 -7.3770898	1983; 1984; 1994; 1999; 2010	N/A	Yes
West Loch Ollay	WLO	57.268033 -7.4151713	1940; 1951; 1994; 2001	2004; 2010	Yes

2.2.3 Phase 3: Paleoecological research

Two sites from which *N. flexilis* has been lost over the past century were chosen for paleolimnological investigation based upon the availability of documentary records: Esthwaite Water (Cumbria, England), and the Dunkeld-Blairgowrie Lochs, Perthshire, Scotland.

a) Esthwaite Water

Esthwaite Water is a shallow lake in the English Lake District, located in a sheltered valley between the much larger lakes of Windermere and Coniston Water (Fig. 2.5). It has a surface area of 96 ha, and a mean depth of 6.4 m. The upper portion of its 1701 ha catchment lies on the sandstones of the Poolscar and Yewbank formations, whilst the lower portion rests on the siltstones and mudstones of the Bannisdale formation. 33% of the catchment is covered with mixed deciduous and coniferous woodland, 30% is improved pasture, 22% is unimproved neutral/calcerous grassland, 10% is arable farming, and the remainder is human settlement, open water and heathland (Fuller *et al.*, 2002). Esthwaite Water was designated a SSSI in 1965 on the basis that it supports a diverse macrophyte community (including, at the time of designation, *N. flexilis)* and a well developed hydrosere, several rare invertebrates including the Cladoceran *Alonella exigua*, and locally important birds such as the Great Crested Grebe. Since its initial designation, it has also been declared a Ramsar site and National Nature Reserve. Esthwaite Water is one of the most intensively monitored lakes in the world, with water chemistry monitoring data dating back to the 1940s (Talling and Heaney, 1988). Based on its nutrient levels in 1940, it is classified as "moderate alkalinity/mesotrophic" in UK legislature, and "oligotrophic to mesotrophic" in the EU Habitats Directive (Hughes *et al.*, 2004). However, it is the most nutrient enriched lake in the English Lake District, with an annual phosphorus loading of 747kg TP (Maberley *et al.*, 2011). This nutrient loading is a result of discharges from agriculture, a fish farm, and from the Hawkshead sewage treatment works, which opened in 1973 (Dong *et al.*, 2012). A benthic core taken

from the northern deep basin of Esthwaite Water in 2006 was analysed for diatoms by Dong *et al.* (2011; 2012), and showed that significant changes in the diatom flora have occurred as the result of nutrient enrichment at the site. Management strategies at the site have focussed on the removal of the fish farm in November 2009, and the addition of secondary and tertiary treatment to the Hawkshead sewage treatment works, initially in 1989, with further upgrades made in 2010 (Maberley *et al.*, 2011).

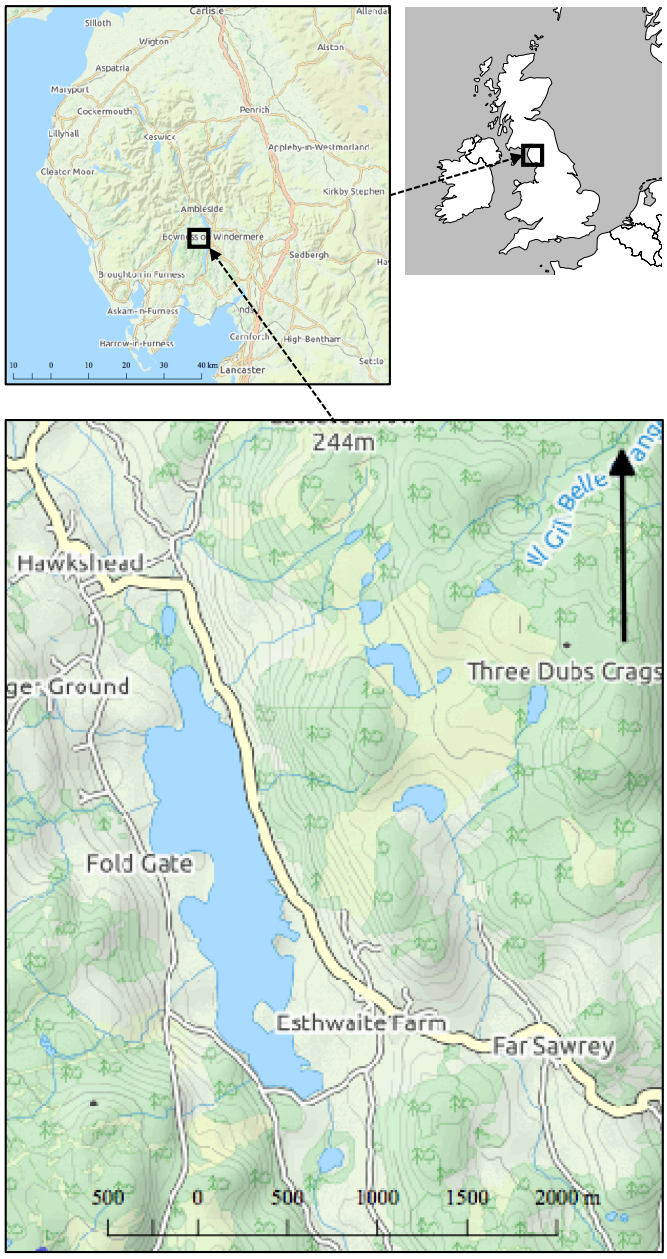

Figure 2.5 Map of Esthwaite Water, Cumbria, England.

The first full macrophyte survey of Esthwaite Water was conducted in 1917 by Pearsall (1920), and has since been surveyed in 1980 (Stokoe, 1983) and 1999 (Darwell, 2000). SCM surveys are also available from 2008, 2011 and 2014. *N. flexilis* was recorded in Esthwaite Water on fifteen separate occasions between 1914 and 1982, but has not been recorded at the site since 1982 (National Biodiversity Network, 2014).

b) The Dunkeld-Blairgowrie Lochs

The Dunkeld-Blairgowrie Lochs are a chain of five shallow, mesotrophic lochs in Perthshire, Scotland, hydrologically linked by the Lunan Burn (Fig. 2.6). Three of these lochs – Loch of Craiglush, Loch of Lowes and Loch of Butterstone, are located within 2km of each other (see Fig. 2.4), whilst Loch of Clunie and Loch of Marlee (also known as Loch of Drumelie) are ~5km and ~7km downstream respectively. The lochs range in surface area from 26 ha (Loch of Craiglush) to 88 ha (Loch of Lowes), with mean depths ranging from 3.4 m (Loch of Butterstone) to 8.9 m (Loch of Marlee and Loch of Clunie). Together, the Dunked-Blairgowrie lochs form a Special Area of Conservation (SAC), designated because of their populations of *N. flexilis.* All five lakes also support otter populations, and Loch of Lowes is managed as a nature reserve by the Scottish Wildlife Trust because it is home to a pair of breeding Ospreys. The lochs lie across the Highland Boundary Fault, with Loch of Craiglush, Loch of Lowes and Loch of Butterstone lying on Dalradian mica-schists, quartzites and semi-pelites, and Loch of Marlee and Loch of Clunie lying on Permian Old Red Sandstones and carboniferous sediments (Walker, 1961). In order to eliminate differences between the lochs caused by the underlying geology, this study focusses only on the upper three lochs. Together, these three lochs have a catchment area of 2226 ha. Catchment land use for the upper three lochs is 35% deciduous and coniferous woodland, 25% improved and unimproved grassland, and 17% dwarf shrub heath, with the remainder comprised of arable farming, bare ground and open water, however much of the catchment of Loch of Craiglush is upland and therefore isolated from the improved grassland and arable farming (Fuller *et al.*, 2002). All three lochs are believed to have been subject to nutrient enrichment from diffuse agricultural

inputs over the course of the twentieth and twenty-first centuries (Ferrier *et al.*, 1997). Until the mid-1990s, a visitors centre owned by the Scottish Wildlife Trust was discharging sewage into Loch of Lowes, with an estimated annual total phosphorus loading of 19.25kg/yr (Ferrier *et al.*, 1997). At Loch of Butterstone, effluent and waste food from six fish cages belonging to the Butterstone Trout Fishery acted as a point source of nutrient enrichment during the period of their operation between 1981 and 2004 (Bennion *et al.*, 2010). Although the cages were removed in 2004, stocking of 150-600 brown and rainbow trout per week is still permitted at the site (Cowx *et al.*, 2012). In deep-water sediment cores taken from Loch of Butterstone and Loch of Lowes in 1998, there is evidence for changes in the diatom flora in line with the timing of nutrient enrichment in the catchments, although a core taken from Loch of Craiglush during the same year did not show any significant changes in diatom flora (Finney, 1998). Further cores taken from Loch of Butterstone in 2009 showed similar changes in the diatom assemblages, with changes in both macrofossil and Cladocera remains in a separate, undated, littoral core also evident (Bennion *et al.*, 2010). In 2004, a catchment management scheme called the "Lunan Natural Care Scheme" was introduced in the catchment, providing financial subsidies to farmers implementing waste management plans (Scottish Natural Heritage, 2004). To date, the ecological impacts of this scheme have not been investigated.

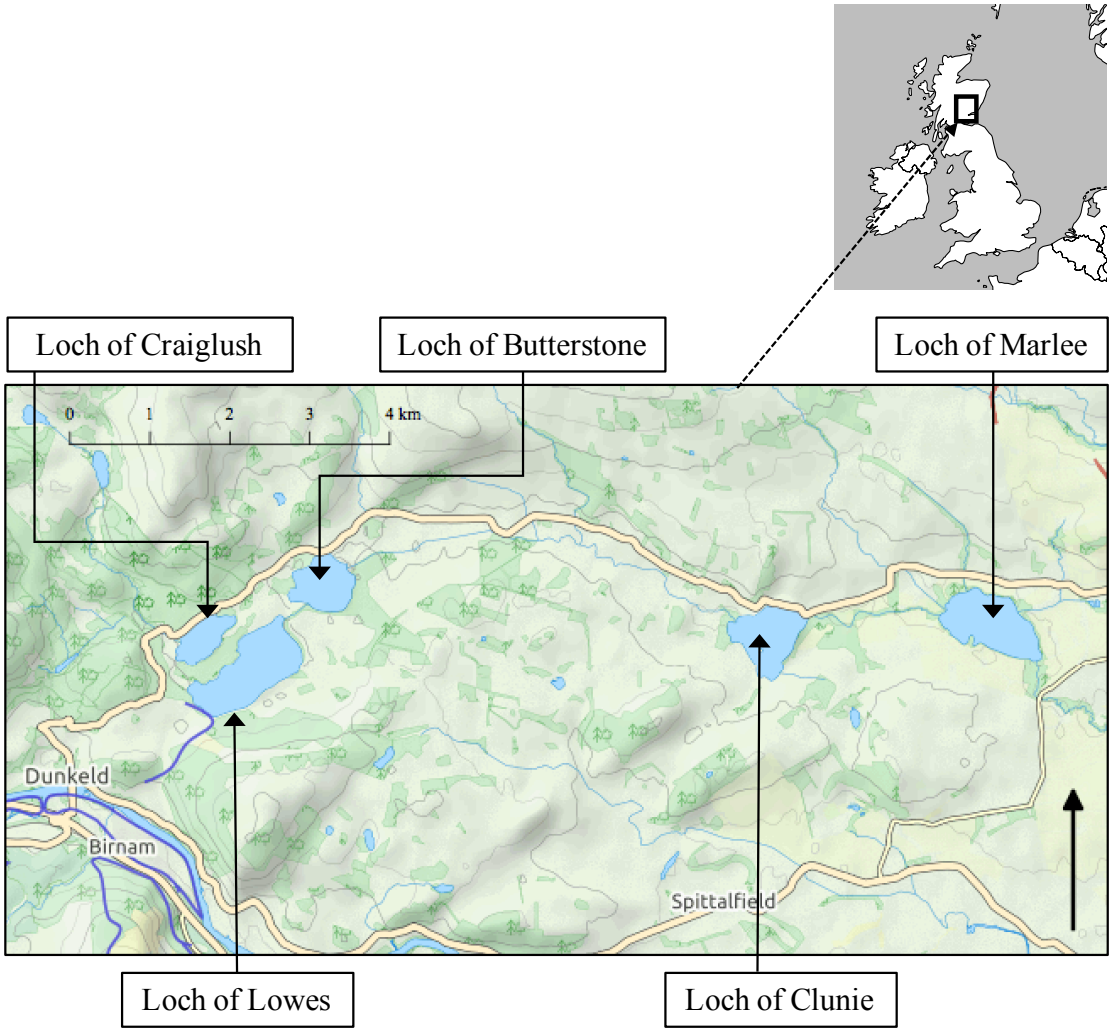

Figure 2.6 Map of the Dunkeld-Blairgowrie Lochs

N. flexilis was first recorded in Loch of Lowes in 1879, in Loch of Craiglush in 1967 and in Loch of Butterstone in 1986 (Scottish Natural Heritage, unpublished data). It has been recorded at all three sites on a regular basis since these dates, including during macrophyte identification courses run by N. Stewart at the sites between 1986 and 2007 (N. Stewart, pers. comms). Following unconfirmed reports that the extent of *N. flexilis* cover was declining at all three lochs, several SCUBA and snorkel surveys of the species were commissioned by Scottish Natural Heritage. The first of these was conducted between 1987 and 1996 using snorkeling, SCUBA and underwater videography, at which time *N. Flexilis* was recorded growing across the entire basin of Loch of Lowes, and growing abundantly in localized patches within Loch of Butterstone and Loch of

Craiglush (James and Barclay, 1996). In 1997, snorkel and SCUBA surveys of Loch of Butterstone and Loch of Craiglush were commissioned by Scottish Natural Heritage, and located extensive but localised beds of *N. flexilis* in both lochs (Dale *et al.*, 1997). All three lochs were again surveyed by SCUBA for *N. flexilis* between 2004 and 2006; during these surveys, the plant was abundant in Loch of Lowes, but only one or two plants were found in each of Loch of Craiglush and Loch of Butterstone (Murphy, 2007, unpublished). Another SCUBA survey of all three lochs was commissioned by Scottish Natural Heritage in 2007, during which the extent of the plant appeared to have expanded in Loch of Lowes, but it was not found at all in Loch of Craiglush or Loch of Butterstone (Benthic Solutions, 2007). *N. flexilis* has not been found in any of the Dunkeld-Blairgowrie Lochs since this 2007 survey.

2.3 Data collection

2.3.1 Phase 1: preliminary development of survey methods and assessment of suitability of paleoecology

Distribution

Macrophyte distribution surveys were carried out at each of the three phase 1 sites in August (Upper Glenastle Loch and Loch of Butterstone in 2013 and Tangy Loch in 2014). *N. flexilis* has a very short, late growth season, so it was important that all sites were surveyed during the last two weeks of August since this is when the plant is most likely to be at its peak distribution (O Connor, 2013). Two types of survey were conducted at each loch: A boat-based survey, and a SCUBA survey. Basic water chemistry readings, alongside a water sample, were collected upon arrival at each site.

Water chemistry

On arrival at each site, Dissolved oxygen (DO), salinity, total dissolved solids (TDS),

conductivity and pH were recorded using a Hach HQ30D meter with CDC401 IntelliCAL and pH101 IntelliCAL probes. Secchi depth was recorded in the middle of the lake using a Secchi disc. Total alkalinity was measured in the field using the standard titration methodology (Wetzel and Likens, 1991). 6 - 8 drops of Bromocresol-green Methyl-red indicator were added to a 100ml sample of lake water, and titrated against 1.6N H_2SO_4 using a Hach digital titrator (model 16900-01) until the sample reached pH 4.5. Alkalinity was then read as mg/L $CaCO_3$. Filtered and unfiltered water samples were collected for later laboratory analysis for total phosphorus (TP), soluble reactive phosphorus (SRP), and nitrate (NO_3) concentrations. TP was measured against standards using the potassium persulphate digestion method, SRP was measured against standards using the ascorbic acid method, and NO_3 was measured against standards using the cadmium reduction method (American Public Health Association, 1989). All laboratory work was undertaken in the water chemistry laboratories at University College London.

Boat-based surveys

Macrophyte surveys largely followed the methodology used at a site of similar size in England by Zhao *et al.* (2006), which compared macrophyte and macrofossil distributions. A gridded sampling pattern was initially laid out on a map of each loch (Fig. 2.7), and grid references were uploaded as waypoints into a Garmin eTrex 10 GPS device. Grid resolution was based on the optimum balance between maximum survey coverage and length of time available; 30 m x 30 m at Upper Glenastle Loch (n = 147), 45 m x 45 m at Tangy Loch (n = 95) and Loch of Butterstone (n = 100). Grid reference, wind exposure, water depth, wind exposure, and percentage cover of each plant species present were recorded at each point. Water depth was measured using a plumb line, whilst grid reference was recorded using the GPS device. Plant percentage cover was estimated from a boat, using a bathyscope assisted by two throws of a double headed rake. Macrophytes were identified to species level using Haslam *et al.* (1982), and, in challenging cases, voucher specimens were collected and sent to expert Nick Stewart for verification. Likely disturbance from wind at each transect was represented as exposure,

and measured using the same method used by Wingfield (2002). This was a simplification of the formula originally used by Keddy (1982) and Weisner (1987):

$$E = \sum_{i=1}^{8} exceedence_{45i^{o}} \times fetch_{45i}$$

where E = exposure, fetch is the distance in km from the nearest shoreline (measured on an aerial photograph) and exceedance is the number of times the wind speed was greater than 12mph. Because wind data was not available for every site, exceedance was simplified to a score of 2 for westerly directions (the predominant wind direction in Scotland) and 1 for all other directions. This method assumes that all sites experience the same wind force, which is not true – some lochs occur right next to the coast, whilst others are sheltered in dips in the landscape. However, in the absence of detailed meteorological records, this method is considered to give an approximation of differences in exposure at each sample point.

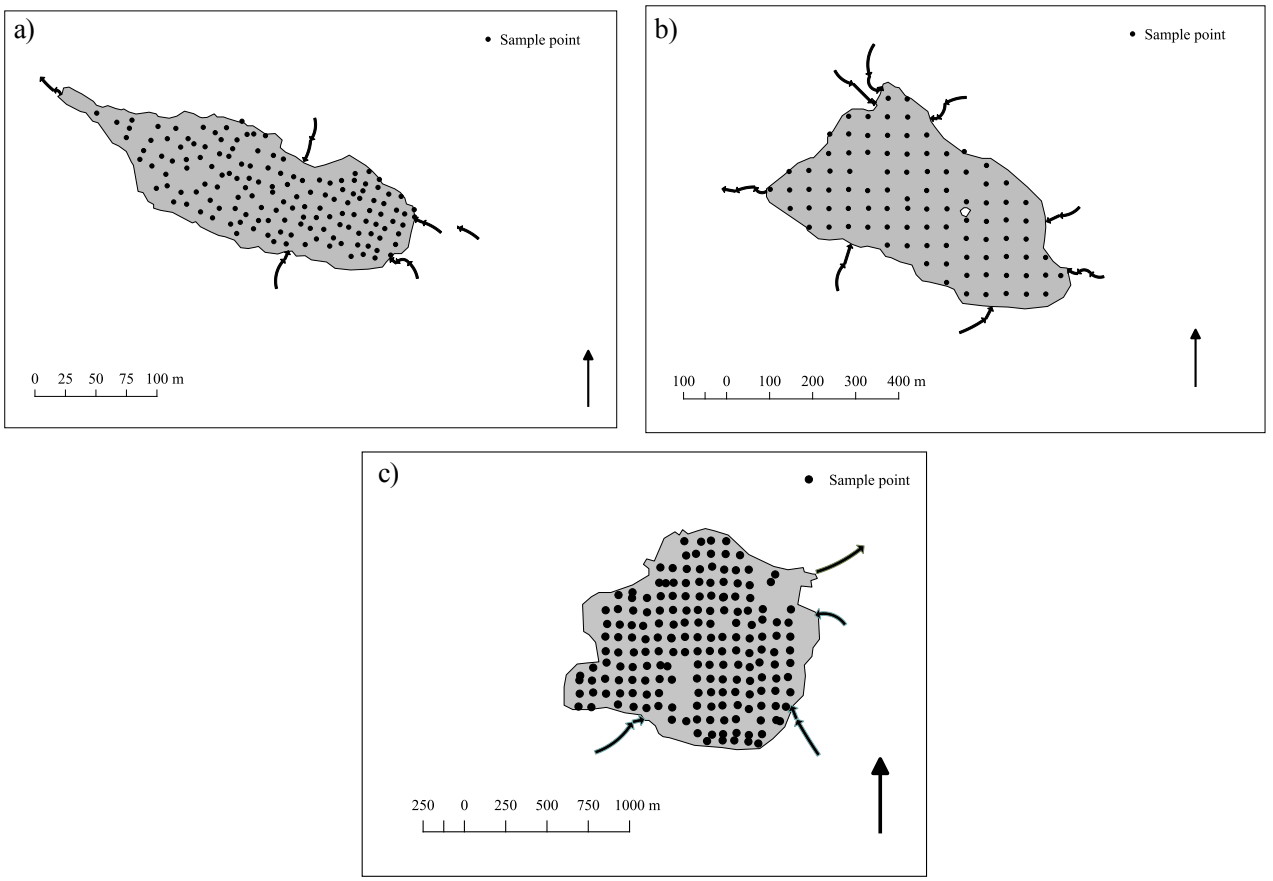

Figure 2.7 Gridded sampling patterns at a) Glenastle Loch, b) Loch Tangy and c) Loch of Butterstone.

At each sample point in the boat survey, the predominant substrate type of each sample point was established either using a bathyscope, grapnel, or Ekman grab. Where more than one substrate type was noted, the first type listed was taken to be the predominant type. The use of SCUBA facilitated quantitative measurement of sediment fluidity. This was measured at every SCUBA sample point using a terrestrial pocket TorVane that was modified for use in the more fluid sediments of the aquatic environment through the addition of four vanes of 6cm diameter and 20cm length (Fig. 2.8). This approach has been successfully used in the estuarine and marine environments by Vickers (1983) and Olafsson and Paterson (2004), and also in freshwater lakes (Schutten *et al.*, 2005). The vane was turned in the sediment, and the torque required to trigger sediment failure was recorded. The following formula was used to calculate shear stress:

$$T = F \div A$$

where T = shear stress, F = force applied and A = cross-sectional area of material (in this case 0.012m^2), with area parallel to the applied force vector.

Figure 2.8 TorVane in use in Loch Ballyhaugh. Photograph by author.

SCUBA surveys

For the phase 1 studies, SCUBA was chosen as an in-water sampling method because it allowed direct observation of submerged macrophytes without the need for the surveyer to surface, prolonging the time available for macrophyte identification. At Glenastle Loch, The SCUBA survey was based upon a transect approach. Eight north-south transects were laid onto a map of the site (Fig. 2.9). These transects were uploaded to a Garmin handheld GPS device as "routes". The GPS device was sealed in a waterproof bag, and two SCUBA divers followed the transects. They recorded depth and *N. flexilis* percentage cover at regular intervals (defined as 3 fin kicks by the diver, and including both areas where *N. flexilis* was present and absent), as well as noting the depth and GPS co-ordinates of the start and end of each stand of *N. flexilis* they came across along the

transect. Percentage cover was estimated over an area of $1m^2$ by eye, using a $1m^2$ quadrat for assistance where appropriate. Whilst transects ensure coverage of the full range of floristic variation over an environmental gradient (in this case water depth), quantitative analysis of the data collected Glenastle Loch was challenging. Therefore, at Tangy Loch, which was visited the following year, the SCUBA survey was based upon a sample point approach. At each of the sample points used in the conventional macrophyte survey, a weighted 1 m^2 quadrat was sunk and divers "hovered" over it, estimating the percentage cover of *N. flexilis* within the quadrat. Point samples have the advantage of providing quantitative results for species density, cover, frequency and/or biomass (Wheater *et al.*, 2011). At Loch of Butterstone, *N. flexilis* was not found during the boat survey. Visibility at the site to SCUBA divers was severely limited by an algal bloom. In order to make the most efficient use of limited time at the site, SCUBA surveys were postponed until 2014. SCUBA surveys focussed on the areas in which *N. flexilis* seeds were found in the largest numbers in the surface sediments, since it was assumed that this was an indicator of nearby plant presence. However, no *N. flexilis* plants were found during the SCUBA survey of Loch of Butterstone.

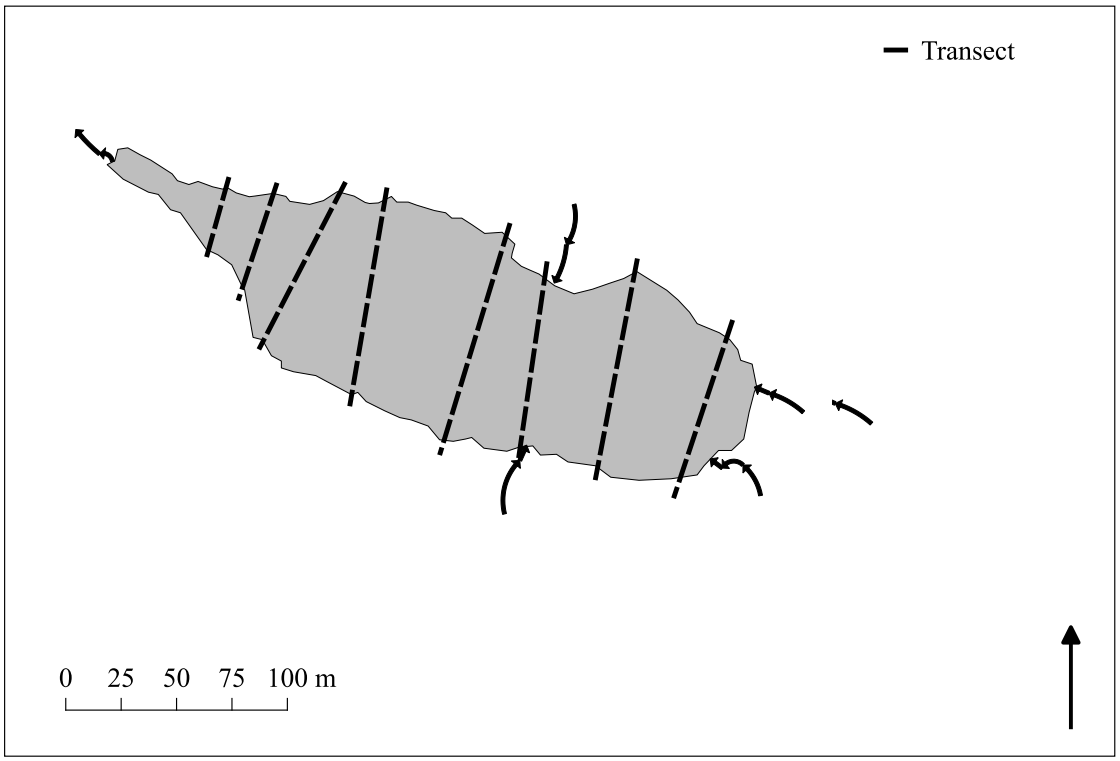

Figure 2.9 Map showing locations of SCUBA transects at Glenastle Loch

Distribution of macrofossils in surface sediments

A surface sediment sample was taken at each point in Glenastle Loch, Loch Tangy and Loch of Butterstone following the methodology used by Birks (1973), using an Ekman Grab, which quickly provided a bulk sample of the top 7 – 10 cm of sediment. This allowed a large volume of sediment to be collected rapidly, and was considered important because previous studies have suggested that sediment volumes of approximately 150cm^3 are optimal for macrofossil analysis (Patmore *et al*. 2014). Furthermore, *N. flexilis* seeds have been shown to germinate and grow when buried with up to 10 cm of sediment, and samples collected in the Ekman Grab could therefore still represent present plant growth (Wingfield 2002). Birks (1973) describes carefully spooning the top few centimetres of mud from the Ekman sample, but this was not possible at any of the sites in this study due to the unconsolidated nature of the sediments collected. Therefore, to ensure that this approach was not skewed by past *N. flexilis* seed distributions, three Glew cores of 69.5

mm diameter (Glew, 1991) ("BUTT9", "BUTT10" and "BUTT11") were additionally taken at Loch of Butterstone (Fig. 2.10), the top 15 cm of which were extruded at 1cm intervals for analysis. These cores were taken in areas where high numbers of seeds were found in the Ekman grabs, and in areas where *N. flexilis* plants were found historically. Glew cores were not taken at Upper Glenastle Loch or Loch Tangy because, unlike at Loch of Butterstone (where *N. flexilis* had recently become extinct), distinction between the numbers of seeds at the top and bottom of the Glew cores was not considered likely.

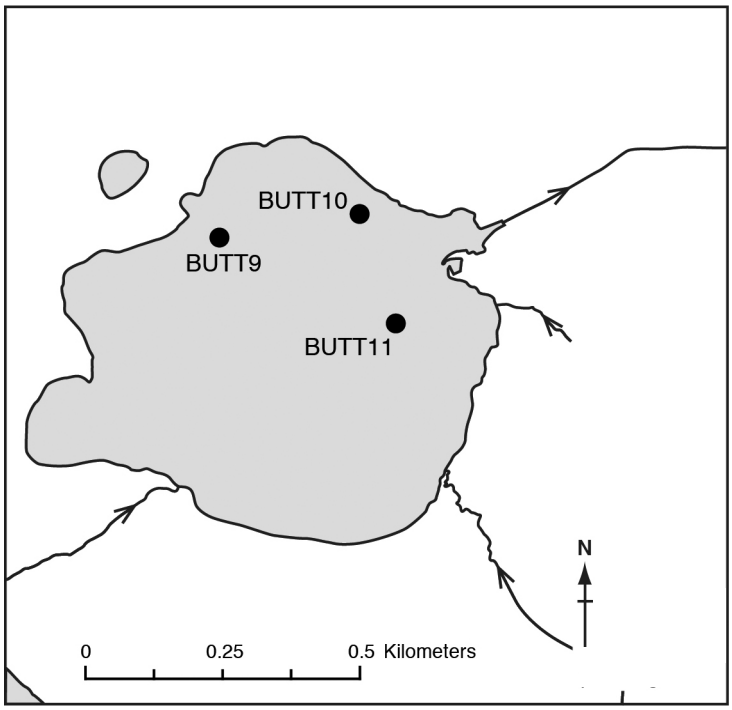

Figure 2.10 Location of Glew cores BUTT9, BUTT10 and BUTT11.

A measured volume of approximately 100 cm^3 wet sediment from each sample, including the extruded Glew cores, was passed through a 355 µm sieve. The retent was transferred into a beaker with some tap water, and in some cases disaggregated using 3 ml of $Na_6O_{18}P_6$. Samples were then examined under a Leica dissecting microscope with a 10x magnification setting, and the number of *N. flexilis* seeds counted.

2.3.2 Phase 2: Further development of sampling method, and plant distribution surveys

The main sources of data for analysis of the current habitat preferences of *N. flexilis* were: a) the results of past SCM surveys held by SNH; b) the results of the pilot studies described above; c) SCM surveys carried out in 2016. In 2016, macrophyte surveys were conducted at 21 of the 26 Scottish *N. flexilis* sites subject to SCM. Surveys were conducted between late July and September 2016, alongside planned SCM surveys carried out by environmental consultancy ECUS on behalf of Scottish Natural Heritage. Past SCMs as well as those carried out in 2016 followed the SCM Guidance for freshwater lakes, which outlines the current methodologies recommended for SCMs (Gunn *et al.*, 2010). The guidance for habitat and community surveys is extremely detailed, and designed to collect as much information about each lake as possible with realistic limitations on time and money spent on each survey. Four sectors of 100m length were marked along the shore. These sectors were chosen to best represent the full diversity of habitats present in the lake. Each sector was split into five 20m sections, transects were marked perpendicular to the shore at the ends of each section, and macrophyte presence and abundance were recorded bathyscope and grapnel at depths of 0.25m, 0.5m, 0.75m and maximum wader depth along transects running perpendicular to the shore at the end of each 20m section. This created the survey pattern shown in Fig. 2.11a. Macrophyte abundance was recorded on a semi-quantitative scale, with "1" representing a species percentage cover <25%, "2" representing 25 – 75% cover, and "3" representing >75% cover. It was also possible to assign this scale to the results of pilot surveys from Upper Glenastle Loch, Loch of Butterstone and Loch Tangy, since the species percentage cover of each sample point was recorded. Thus, SCM survey results gave the species composition of every sample point in each survey, enabling analysis of species compositions to be made on a micro-habitat scale for comparison to spatially heterogeneous variables like sediment type. To analyse *N. flexilis* on a whole-lake scale (for example, in comparison to site water chemistry), the number of sample points that contained *N. flexilis* in each survey was used to represent site *N. flexilis* abundance.

At each SCM sample point, water depth, wind exposure, and sediment type were

recorded using the same methods employed in the pilot surveys. Sediment fluidity was not recorded, since this was not possible in deep water without the use of SCUBA equipment. Water chemistry data were not recorded. Instead, it was obtained from the Scottish Environmental Protection Agency (SEPA) monitoring program where available (pers. comms). For most sites, this consisted of between one and eight annual measurements of total alkalinity, conductivity, pH, dissolved oxygen, salinity, total suspended solids, light extinction coefficient (LEC – an inverse function of Secchi depth) and total phosphorus and nitrate concentrations. The use of monitoring records was considered more reliable than one-off measurements taken in the field on the day of the survey.

At sites where *N. flexilis* is present, additional monitoring is usually required as part of the SCM to ensure that the species is adequately captured by the survey. It is suggested that two distinct 'populations' of the species are identified and recorded at each site, and the presence/absence of the species is recorded in 20 random quadrats within each 'population' (Interagency Freshwater Group, 2015). In the case of plants that occupy the deeper water, snorkel and/or SCUBA surveys are recommended as an alternative strategy that allows direct observation of the lakebed (Gunn *et al.*, 2004). Aside from this, there is little guidance given on how to conduct these surveys. Following the two pilot studies, it was concluded that, at the water depths in which *N. flexilis* occurs, SCUBA equipment was unnecessary. Therefore, snorkels were used for the 2016 SCM surveys for *N. flexilis*. A snorkeler swam in a zig-zag pattern along the length of each 100m sector from the shore to the maximum vegetated depth (Fig. 2.121b). Once a population of *N. flexilis* had been found, the snorkeler recorded the percentage cover of *N. flexilis* at 20 random quadrats within the 100m sector, in line with standard SCM practice. Additionally, the snorkeler also recorded the minimum and maximum depths of *N. flexilis* colonisation and made a very rough assessment (by eye) of the average percentage cover of *N. flexilis* within this depth zone across the 100m sector. Any large changes in estimated *N. flexilis* percentage cover between different 20m sections of the 100m sector were noted. A sketch was made of the changing macrophyte species assemblages with depth, and sediment types were also noted on this sketch.

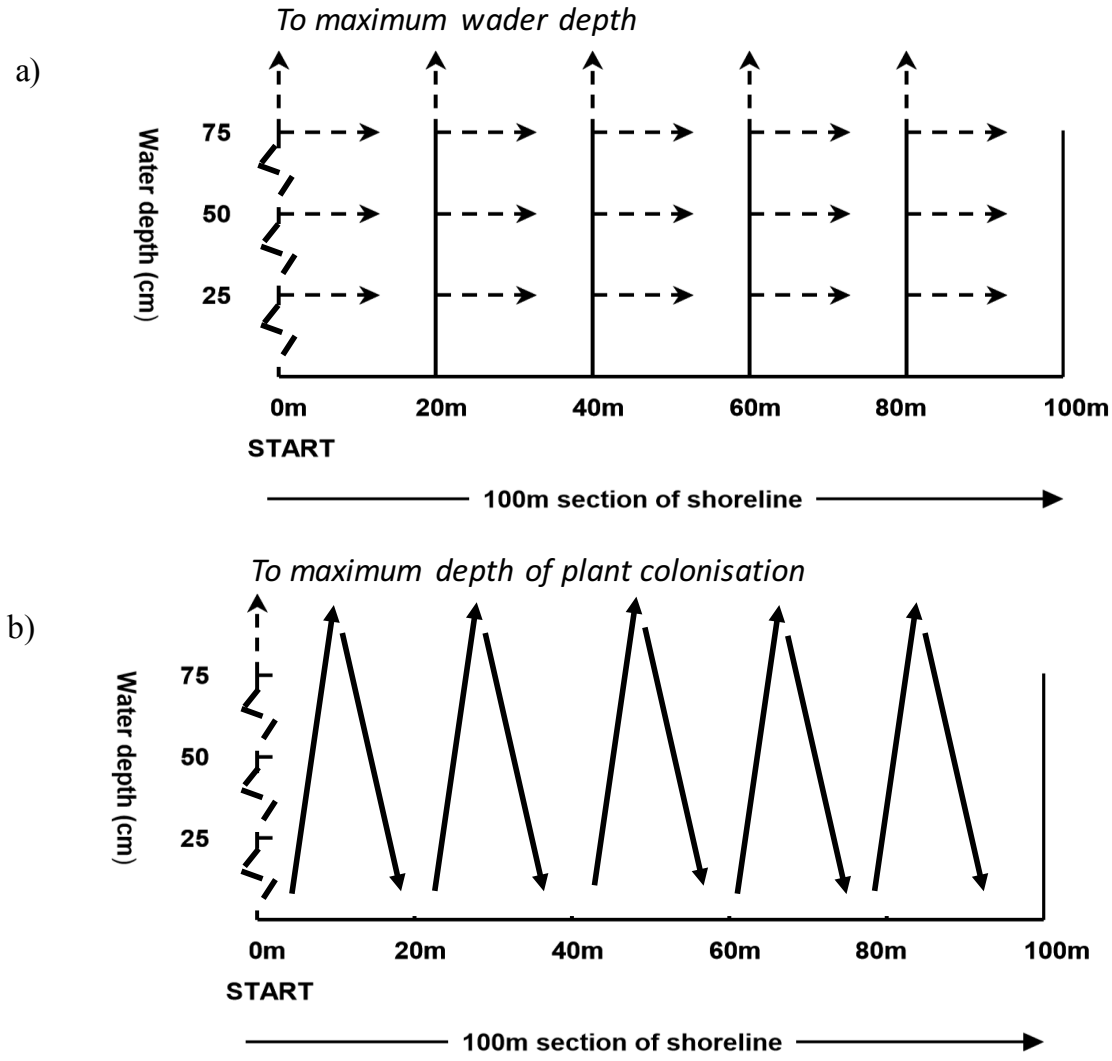

Figure 2.11 Macrophyte sampling strategies for SCMs. a) Transect-based sampling strategy, from Gunn et al (2010); b) zig-zag sampling strategy for snorkel sectors.

2.3.3 Phase 3: Paleoecological research

Primary data

Large diameter piston cores were taken from the littoral zones of Esthwaite Water (ESTH9), Loch of Lowes (LOWIB) and Loch of Craiglush (CRAIB) using the "Big Ben"

wide-diameter piston corer (Patmore *et al.*, 2014) in 2013. The cores were extruded in the field at 1cm intervals, and note was made of any visual stratigraphic changes. The percentage loss on ignition (LOI) and percentage carbonate were analysed following cooking of the sediments at 550^{o}C and 950^{o}C respectively, following standard methods (Dean, 1974). Changes in sediment colouration were described via comparison to Munsell soil charts (Munsell Colour (Firm), 2010). Descriptions of all cores are shown in Table 2.2, with cores from which secondary data were derived included for completeness (see page 66). Fig. 2.12 shows coring locations for all cores used in this study.

*Table 2.2 Core descriptions. *LOWIB did not date, and was therefore excluded from further study (see page 65)*

Core	Date	Latitude/ Longitude	Water depth (m)	Core type	Core length (m)	Extrusion intervals
ESTH9	1/9/2013	54.3612/ -2.9907	1.5	Big Ben	1.09	1cm
LOWIB*	24/8/2014	56.5783/ -3.5397	1.45	Big Ben	0.19	1cm
CRAIB	25/8/2014	56.5828/ -3.5536	1.1	Big Ben	0.56	1cm
Secondary data from Dong et al (2011; 2012) – see page 66						
ESTH1	7/6/1995	54.3639/ -2.9900	15	Mackereth corer	0.86	1cm
ESTH7	12/4/2006	54.3639/ -2.9900	15	Percussion piston corer	0.80	0.5cm

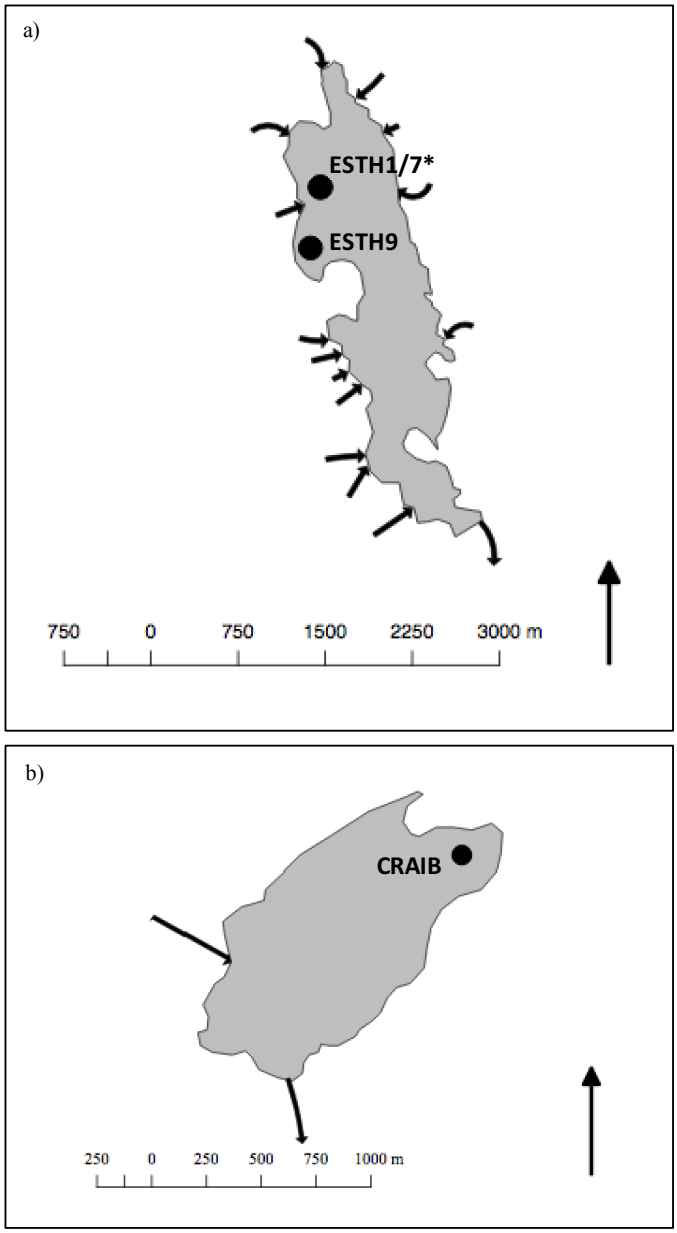

*Figure 2.12 Coring locations at a) Esthwaite Water and b) Loch of Craiglush. *ESTH1/7 is a core from which secondary data was derived (see page 66), and is included here for completeness.*

The cores were radiometrically dated in the Environmental Radiometric Facility at University College London, where they were analysed for ^{210}Pb, ^{226}Ra, ^{137}Cs and ^{241}Am by direct gamma assay using ORTEC HPGe GWL series well-type coaxial low background intrinsic germanium detector. Lead-210 was determined via its gamma

emissions at 46.5keV, and ^{226}Ra by the 295keV and 352keV gamma rays emitted by its daughter isotope ^{214}Pb following three weeks storage in sealed containers to allow radioactive equilibration. Cesium-137 and ^{241}Am were measured by their emissions at 662kev and 59.5kev (Appleby *et al.*, 1986). The absolute efficiencies of the detector were determined using calibrated sources and sediment samples of known activity. Corrections were made for the effect of self-absorption of low energy gamma rays within the sample (Appleby *et al.*, 1992). In both cores, variations in unsupported ^{210}Pb were non-monotonic, which precluded the use of the constant initial concentration (CIC) dating model (Appleby, 2001). The simple constant rate of ^{210}Pb supply (CRS) dating model was therefore used. The LOWIB sediment core was not successfully dated due to uneven sedimentation rates, and LOWIB was therefore not studied further.

From each of the 1cm intervals that were dated between 1850 and present, measured samples of approximately 50 - 75cm^3 (determined by water displacement) were sieved at 350 and 125 μm, following the methodology described by Birks (2001). The residues were examined at x10 – x40 magnification under a Leica light microscope, and macrofossil remains (e.g. seeds, leaf spines, leaf tips, insect carapaces, bryozoan statoblasts) were identified and counted. Identification was made by comparison with reference material held at the Environmental Change Research Centre, University College London. The results for each species (or species group where identification to species level was not possible) were expressed as number of remains per 100 cm^3 wet sediment. In order to gain further insight into ecological changes affecting the rest of the lake ecosystem, analysis for chitinous cladoceran remains was carried out on every other sample (i.e. at intervals of 2cm down the sediment core), following the techniques described by Korhola and Rautio (2001). Cladocerans are particularly useful in conjunction with macrofossil analysis, because they reflect the presence of macrophyte habitats within a lake (Davidson *et al.*, 2010). Sediments were air dried, and samples weighing at least 1g were heated in 10% KOH (a deflocculating agent) for 20 minutes, magnetically stirred, and passed though a 25μm mesh sieve. A sub-sample was then stained using safranin and examined under a Leica light microscope. Remains were identified with reference to Szeroczyńska and Sarmaja-Korjonen (2007), and expressed

in terms of percentage relative abundance.

Secondary Data

a) Existing sediment cores

In addition to the cores collected as part of this PhD, data from previous cores were also used. These cores were ESTH1 and ESTH7 (Dong *et al.*, 2011; Dong *et al.*, 2012, described in Table 2.2). These are pelagic cores (Fig. 2.12), and have been previously analysed for diatoms. They were dated using the radiometric methods described above, also in the Environmental Radiometric Facility at University College London. Diatom analysis followed the standard methodology (Battarbee *et al.*, 2001). H_2O_2 was added to the dried sediment samples and suspended in a water bath, which was slowly raised to a temperature of 80^0C for two to four hours, to remove organic and mineral matter. Two drops of 50% HCl were added to get rid of any remaining carbonates. Samples were centrifuged at 1200rpm for four minutes, left to settle, and the supernatant removed, before this washing process was repeated. Samples were diluted to a concentration of what, transferred by pipette to a glass slide, and mounted using Naphrax. A minimum of 500 valves per slide were identified under a light microscope at x1000 magnification.

b) Environmental monitoring data

Long term monitoring data (alkalinity, pH, TP, PO4, NO3, NH4, DO, Secchi depth, and surface water temperature), have been collected fortnightly from Esthwaite Water since 1945 by the Freshwater Biological Association (FBA) and the Centre for Ecology and Hydrology (CEH) (Maberly *et al.*, 2017). Measurements were taken from a boat at the deepest point in the lake, and based on an integrated water sample from 0-5m. Water samples were analysed at the FBA and CEH laboratories.

Water quality at the Dunkeld-Blairgowrie lochs has been monitored by SEPA in relation

to a catchment management scheme since 1987 (SEPA, unpublished data). Water samples are taken monthly from the inlet to Loch of Craiglush and the outlets of Loch of Lowes and Loch of Butterstone. Water samples were analysed at the SEPA laboratories for alkalinity, pH, TP, SRP, total nitrogen, NO2, NH3, DO, biological oxygen demand (BOD), conductivity, suspended solids, and surface water temperature.

2.4 Data analysis

Unless otherwise stated, all statistical analysis in this PhD was carried out using R 'stats' package version 2.15.3, in the software RStudio (R Core Team, 2013; RStudio Team, 2016). Before statistical analysis, all datasets were tested for normality using a Shapiro-Wilk's test (Shapiro and Wilk, 1965), and subsequent choice of analytical methodologies were based upon the results of this test.

The spatial distributions of *N. flexilis* recorded using both boat-based methods and SCUBA at all three sites were mapped using QGIS (QGIS Development Team, 2016). Differences between the mean *N. flexilis* percentage cover and *N. flexilis* depth range surveyed using boat-based methods and SCUBA/snorkel were compared using non-parametric Mann-Whitney tests (Mann and Whitney, 1947). Correlations between *N. flexilis* minimum, maximum and median recorded depths and measured water chemistry variables were tested using Kendall's tau correlation co-efficient, which was considered the best choice of non-parametric test based on its suitability for small and non-monotonic datasets (Kendall, 1938).

To assess the lake-wide conditions that affect *N. flexilis* presence, *N. flexilis* abundance and site species composition were represented as an estimate of species percentage cover. This was done by calculating the percentage of sample points in each survey that contained each species. To assess conditions on a micro-habitat scale, each sample point within the surveys was treated on an individual basis, creating a total of 4168 individual data points. Correlations between water chemistry variables, lake-wide *N. flexilis* abundance and lake-wide *Elodea* spp. abundance were tested using Kendall's tau

correlation coefficient. Differences between groups of sites or samples in which *N. flexilis* and/or *Elodea* spp. were present or absent were investigated using student's t-tests (parametric test) and Mann-Whitney tests (non-parametric test) for differences of means. Pearson's chi-squared tests for association were used to investigate differences in the categorical substrate data. Whilst none of these statistical methods specifically considered the effects of spatial auto-correlation, it was assumed that any such auto-correlation would be a factor of water depth, which was explored as a variable in its own right. Associations between species at both lake-wide and micro-habitat scales were investigated using ordination methods, conducted using the "vegan" R package (Oksanen *et al.*, 2017). Initially, detrended correspondence analyses were conducted. In cases where the gradient length of axis 1 was less than 4.0sd, species distributions were assumed to be linear and Principal Components Analysis (PCA) was used. Where gradient lengths were greater than 4.0sd, distributions were assumed to be unimodal and Correspondence Analysis (CA) methods were used. Two-way indicator species analysis (TWINSPAN; Hill, 1979) – a form of cluster analysis designed to classify vegetation samples and species – was used to group sites by their macrophyte species composition. This allowed the identification of different lake types and species associations within the full set of sites included in the study. The WinTwins software was used to conduct the analysis (Hill and Šmilauer, 2005).

Distributions of *N. flexilis* plants and seeds were analysed within sites and across all three sites, and, at Loch of Butterstone, were compared to the historical plant distribution studies of Stewart (1982, pers. commun.), James and Barclay (1996, unpublished) and Murphy (2007). Relationships between seed numbers and *N. flexilis* percentage cover, total percentage cover, water depth, latitude and longitude were assessed on a site-by-site basis, and plotted on scatter charts to assess normality and monotony. Subsequently, Kendall's tau was used to assess correlation between these variables. Mann-Whitney U tests were used to compare *N. flexilis* plant and seed numbers between lakes. Kernel density analysis (a non-parametric estimation of the probability density function, based upon assigning each individual sample to its parent site and then using site as the exploratory variable) was used to estimate the probability that a sediment sample with a

set number of seeds was taken from each site. Based on these kernel density plots, and supplemented with information from the historical botanical records, the sediment cores from Esthwaite Water and Loch of Craiglush were split into depth zones with relation to their predicted *N. flexilis* abundance. These zones were plotted onto stratigraphic diagrams showing changes in species assemblages, allowing changes to be interpreted with direct reference to the estimated size of the *N. flexilis* populations. Associations between *N. flexilis* and other remains were investigated using the ordination methods described above.

3. Towards an improved protocol for monitoring inter-annual changes in *N. flexilis* populations

3.1 Introduction

This chapter aims to establish an improved protocol for monitoring inter-annual changes in *N. flexilis* populations. It asks the following questions:

1. Are SCUBA and snorkel surveys for *N. flexilis* more accurate than grapnel surveys?
2. Where is *N. flexilis* most likely to be found in a loch, based on variables that are readily available to surveyors? What area of a lake must be surveyed to stand the best chance of finding *N. flexilis*?
3. Can this information be used to improve current SCM protocols to make them more suitable for tracking changes in *N. flexilis* populations over time?

Initially, two study sites were selected from a database containing all the existing *N. flexilis* records for UK lakes over the last 150 years: Glenastle Loch (OS grid reference NR 300447) and Tangy Loch (NR 695280). These sites were selected because a) recent surveys reported that *N. flexilis* was growing abundantly at both sites and b) both sites were relatively accessible compared to others with known populations of *N. flexilis.* These two lochs were visited in August 2013 and August 2014 respectively, and used as pilot sites to test the effectiveness and practicalities of SCUBA, snorkel and grapnel surveys as well as the relative merits of different sampling strategies. In 2016, the new methodologies were trialed and honed alongside standard SCMs of 21 SSSIs in Scotland. These sites varied in *N. flexilis* abundance, allowing the methods to be tested at sites at which the plant was not abundant. During the 2016 study, basic water chemistry variables were recorded to establish whether they could be used as an aid to locating populations of *N. flexilis.* This differs from the ecological work presented in chapter 4 because it is based strictly on variables that are measured in the field on the day of the survey, rather than being derived from monitoring programs designed to more accurately represent the true water chemistry of the site.

3.2 Results

3.2.1 Are SCUBA and snorkel surveys for *N. flexilis* more accurate than grapnel surveys?

At Glenastle Loch, *N. flexilis* was found at 48% of points sampled by grapnel (n = 147) and 53% of points sampled by SCUBA (n = 133). At Tangy Loch, *N. flexilis* was found in 12% of quadrats by grapnel and at 24% by SCUBA (n = 82). The overall distribution (Fig. 3.1) of *N. flexilis* recorded by each method was similar at both sites, although higher abundances of *N. flexilis* were recorded by SCUBA than by grapnel at the western end of Glenastle Loch. *N. flexilis* was also recorded more frequently by SCUBA than by grapnel in the south-east corner of Tangy Loch. The range of depths at which *N. flexilis* was observed by SCUBA was larger than grapnel surveys at both sites (Table 3.1 and Fig. 3.2). Mann-Whitney tests revealed significant differences between the mean depth at which *N. flexilis* was observed by each method at both sites (Glenastle Loch $W = 2402.5$, $p = 1.67 \times 10^{-8}$; Tangy Loch $W = 185.5$, $p = 0.01$). At Glenastle Loch, *N. flexilis* was also recorded less frequently at shallower depths by SCUBA than by grapnel. However, this sampling bias does not apply to Loch Tangy.

The range of percentage covers of *N. flexilis* observed by SCUBA was also larger than grapnel surveys at both sites, although there was no significant difference between the means of the two groups (Glenastle Loch $W = 1652$, $p = 0.27$; Tangy Loch $W = 75.5$, $p = 0.08$). At Tangy Loch, a larger number of points at which *N. flexilis* abundance was low were recorded by SCUBA than by grapnel. *N. flexilis* was not recorded at Loch of Butterstone by either method.

Table 3.1 Comparisons between minimum, median, maximum and mean depths and N. flexilis *percentage covers recorded by grapnel and SCUBA at Glenastle Loch and Tangy Loch*

		Min.	**Median**	**Max.**	**Mean**
Depth (m)	Glenastle Loch	Grapnel: 0.88 SCUBA: 0.90	Grapnel: 1.36 SCUBA: 1.70	Grapnel: 1.82 SCUBA: 2.20	Grapnel: 1.33 SCUBA: 1.65
	Tangy Loch	Grapnel: 0.08 SCUBA: 0.08	Grapnel: 1.2 SCUBA: 1.48	Grapnel: 1.53 SCUBA: 1.80	Grapnel: 1.11 SCUBA: 1.39
***N. flexilis* percentage cover**	Glenastle Loch	Grapnel: 2 SCUBA: 5	Grapnel: 20 SCUBA: 20	Grapnel: 95 SCUBA: 100	Grapnel: 29 SCUBA: 36
	Tangy Loch	Grapnel: 1 SCUBA: 1	Grapnel: 10 SCUBA: 3.5	Grapnel: 40 SCUBA: 50	Grapnel: 12 SCUBA: 8.5

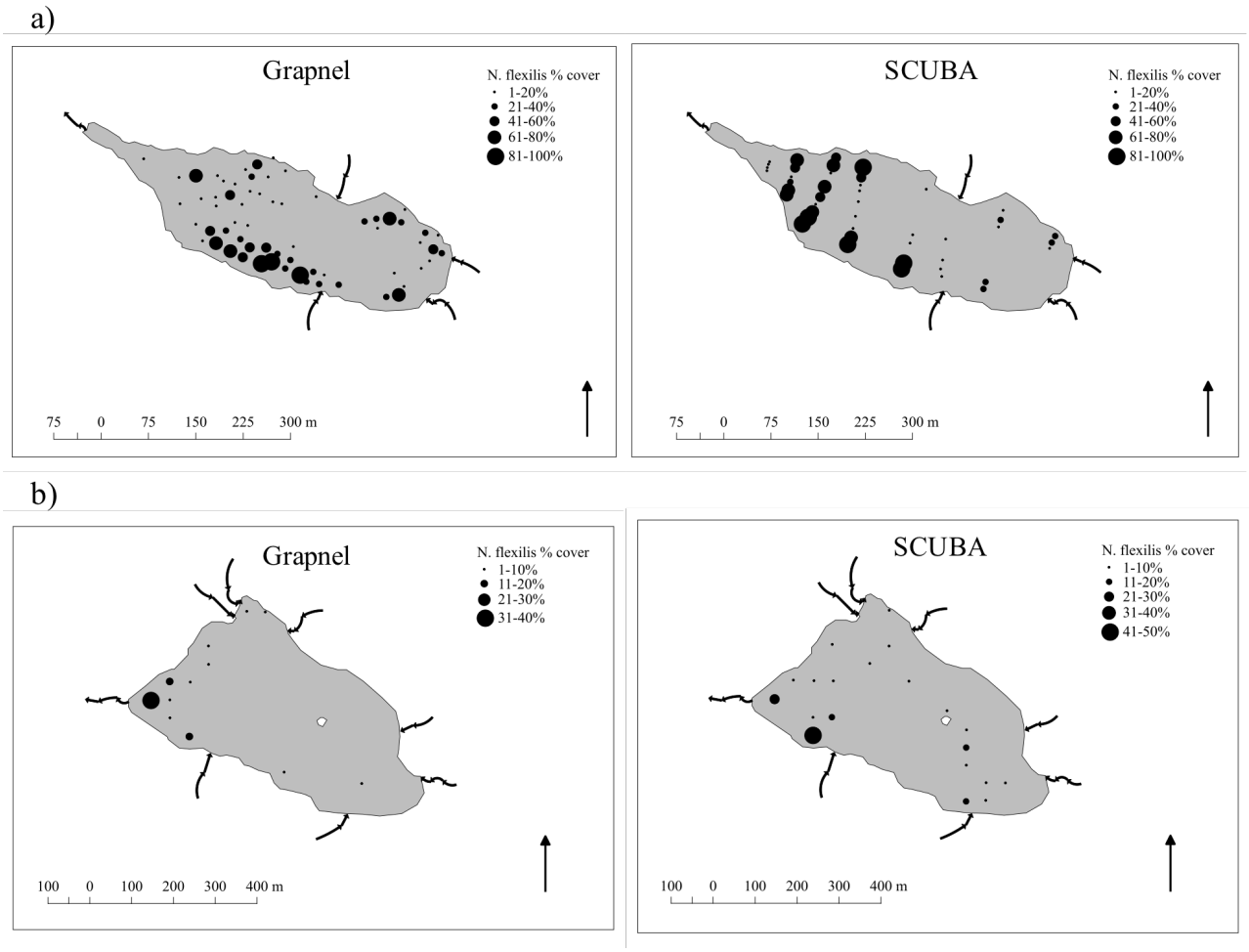

Figure 3.1 Maps comparing distribution of N. flexilis *found by grapnel and SCUBA at a) Glenastle Loch and b) Loch Tangy.*

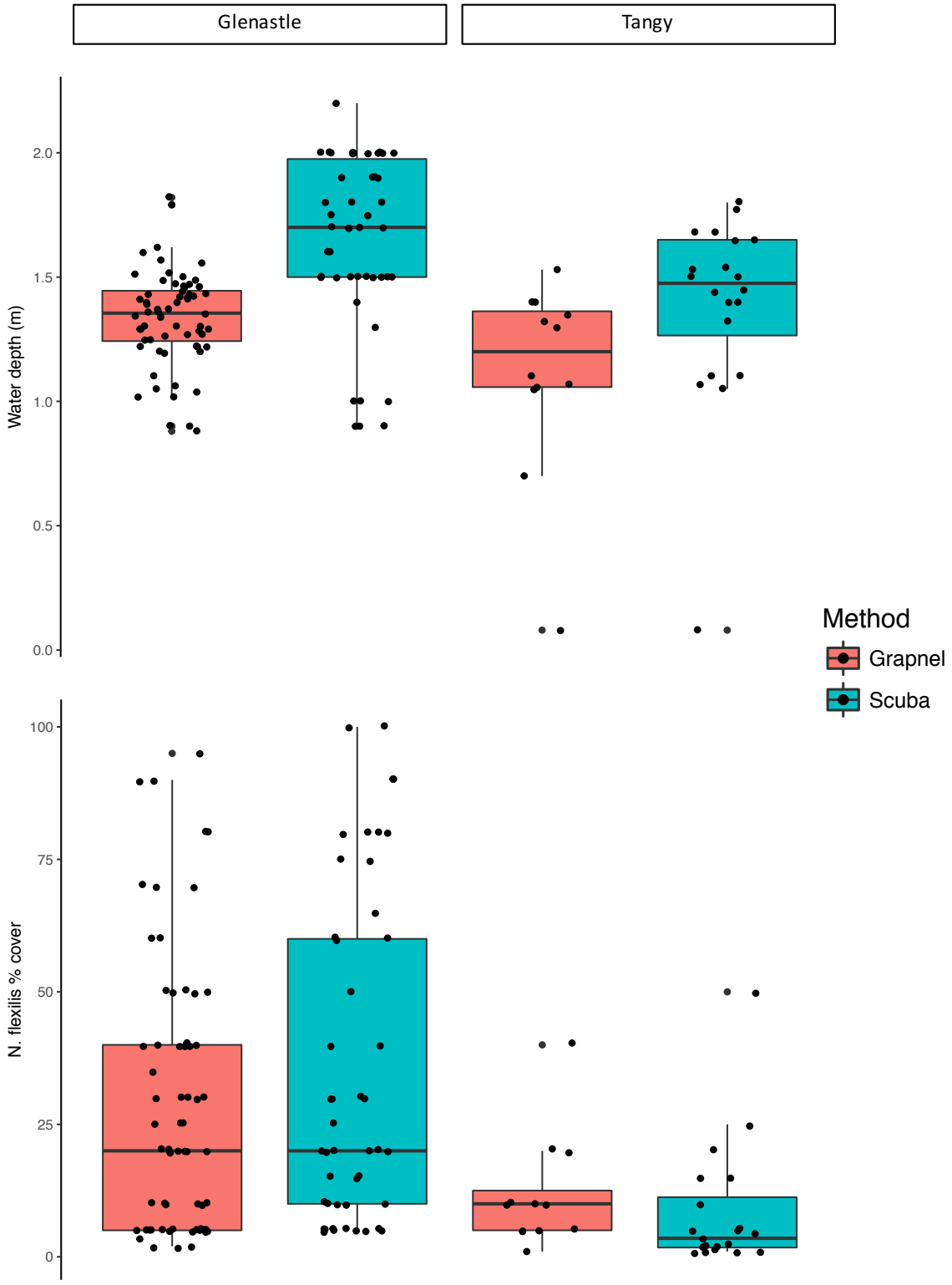

Figure 3.2 Boxplots comparing a) water depth and b) percentage cover of N. flexilis *found using grapnel (red) and SCUBA (blue) in Glenastle Loch and Tangy Loch*

In 2016, *N. flexilis* was found at twelve sites. Two of these sites – Loch Druidibeg and Loch Gherradh Mhic Iain – were not surveyed by snorkel because of the health and safety risk from cold water temperatures. Of the remaining ten sites, *N. flexilis* was found by grapnel at seven sites and by snorkel at all ten sites. At Loch Scarie and Mid Loch Ollay, *N. flexilis* was not found during the grapnel surveys and was only located using a snorkel. At all other sites except Loch Ballyhaugh, *N. flexilis* was found much more frequently by snorkel than by grapnel. At Loch Mhaddaidh, quadrats were not recorded using a snorkel because of diver illness, however the overall *N. flexilis* cover at the site was estimated as abundant during a quick survey swim; quantitative results comparing the two methods are therefore not available for this site. Figs. 3.3 and 3.4 show the water depths and percentage covers of *N. flexilis* found using each method, and the results of the Mann-Whitney tests, alongside minimum, median, maximum and median data for the seven sites at which *N. flexilis* was recorded using both methods are shown in Table 3.2. At Loch Ballyhaugh, Loch nan Cnamh, and Loch na Cuithe Moire, the mean depth of sample points at which *N. flexilis* was recorded by snorkel was significantly greater than by grapnel, whilst at Loch an Eilean *N. flexilis* was recorded at significantly lower mean depths by snorkel than by grapnel. At Loch Nan Cnamh, the mean percentage *N. flexilis* cover recorded by snorkel was higher than that recorded by grapnel, whilst at Loch na Cuithe Moire it was lower. These results are based upon small sample sizes (at Loch Phuirt Ridh, for example, *N. flexilis* was only recorded by grapnel once), and should be treated with appropriate caution.

Table 3.2 Differences between water depth of sample points and N. flexilis *% cover recorded by grapnel (G) and snorkel (S) during 2016 SCMs. Significant differences are shown in bold.*

		n	Min.	Median	Max.	Mean	Test result
Depth (m)	Loch Ballyhaugh	G: 18 S: 16	G: 1.0 S: 1.2	G: 1.3 S: 1.4	G: 1.3 S: 1.5	G: 1.25 S: 1.41	**W = 87.5** **P = 0.05**
	Loch Cuilc	G: 3 S: 5	G: 0.75 S: 0.75	G: 1.0 S: 0.75	G: 1.0 S: 0.75	G: 1.91 S: 0.75	W = 4 P = 0.24
	Loch na Cuithe Moire	G: 7 S: 19	G: 1.0 S: 1.3	G: 1.0 S: 1.4	G: 1.0 S: 2.1	G: 1.0 S: 1.54	**W = 24.5** **P = 0.01**
	Loch an Eilean	G: 10 S: 23	G: 0.75 S: 0.3	G: 1.0 S: 1.9	G: 1.0 S: 1.2	G: 0.93 S: 1.84	**W = 59.5** **P = 0.01**
	Loch Nam Cnamh	G: 3 S: 34	G: 1.0 S: 1.1	G: 1.0 S: 1.6	G: 1.0 S: 1.7	G: 1.0 S: 1.49	**W = 6** **P = 0.01**
	Loch Phuirt Midh	G: 1 S: 33	G: 1.0 S: 0.5	G: 1.0 S: 0.9	G: 1.0 S: 1.8	G: 1.0 S: 0.82	W = 24 P = 0.36
	Schoolhouse Loch	G: 3 S: 30	G: 1.0 S: 0.5	G: 1.0 S: 1.5	G: 1.0 S: 1.8	G: 1.0 S: 1.35	W = 19.5 P = 0.09
N. flexilis percentage cover	Loch Ballyhaugh	G: 18 S: 16	G: 1 S: 1	G: 1.5 S: 2	G: 3 S: 3	G: 1.6 S: 1.9	W = 110 P = 0.21
	Loch Cuilc	G: 3 S: 5	G: 1 S: 1	G: 1 S: 1	G: 1 S: 2	G: 1 S: 1.2	W = 6 P = 0.61

PTO

	Loch na Cuithe Moire	G: 7 S: 19	G: 1 S: 1	G: 3 S: 2	G: 3 S: 3	G: 2.4 S: 1.7	**W = 99.5** **P = 0.04**
	Loch an Eilean	G: 10 S: 23	G: 1 S: 1	G: 1 S: 1	G: 2 S: 3	G: 1.1 S: 1.3	W = 96 P = 0.31
	Loch Nam Cnamh	G: 3 S: 34	G: 1 S: 1	G: 1 S: 3	G: 1 S: 3	G: 1 S: 2.4	**W = 6** **P = 0.01**
	Loch Phuirt Midh	G: 1 S: 33	G: 1 S: 1	G: 1 S: 1	G: 1 S: 3	G: 1 S: 1.6	W = 10 P = 0.49
	Schoolhouse Loch	G: 3 S: 30	G: 1 S: 1	G: 1 S: 2	G: 2 S: 3	G: 1.3 S: 1.8	W = 31 P = 0.36

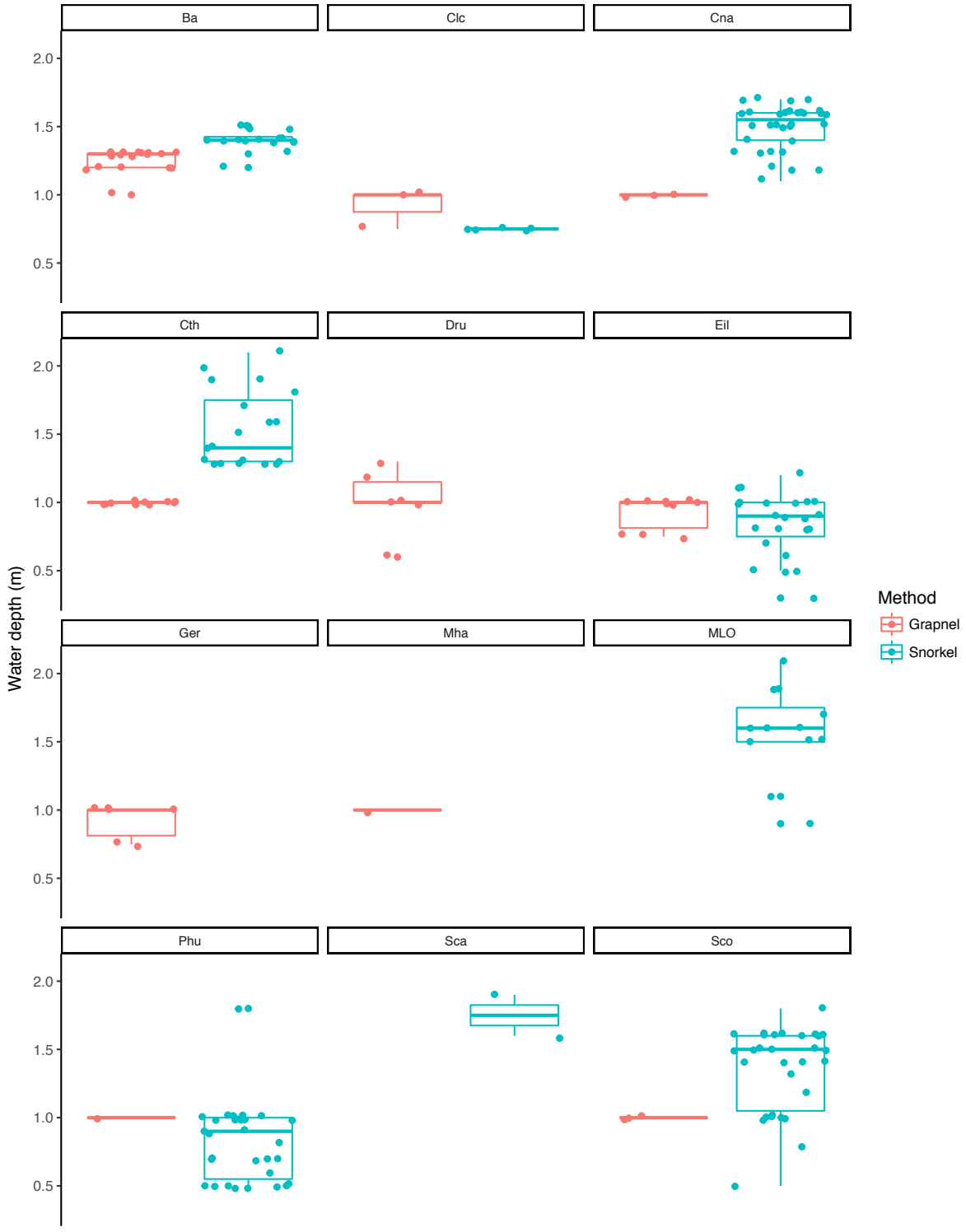

Figure 3.3 Boxplots comparing water depths at which N. flexilis *recorded by grapnel (red) and snorkel (blue) during 2016 SCMs. Site name abbreviations are defined in table 2.1.*

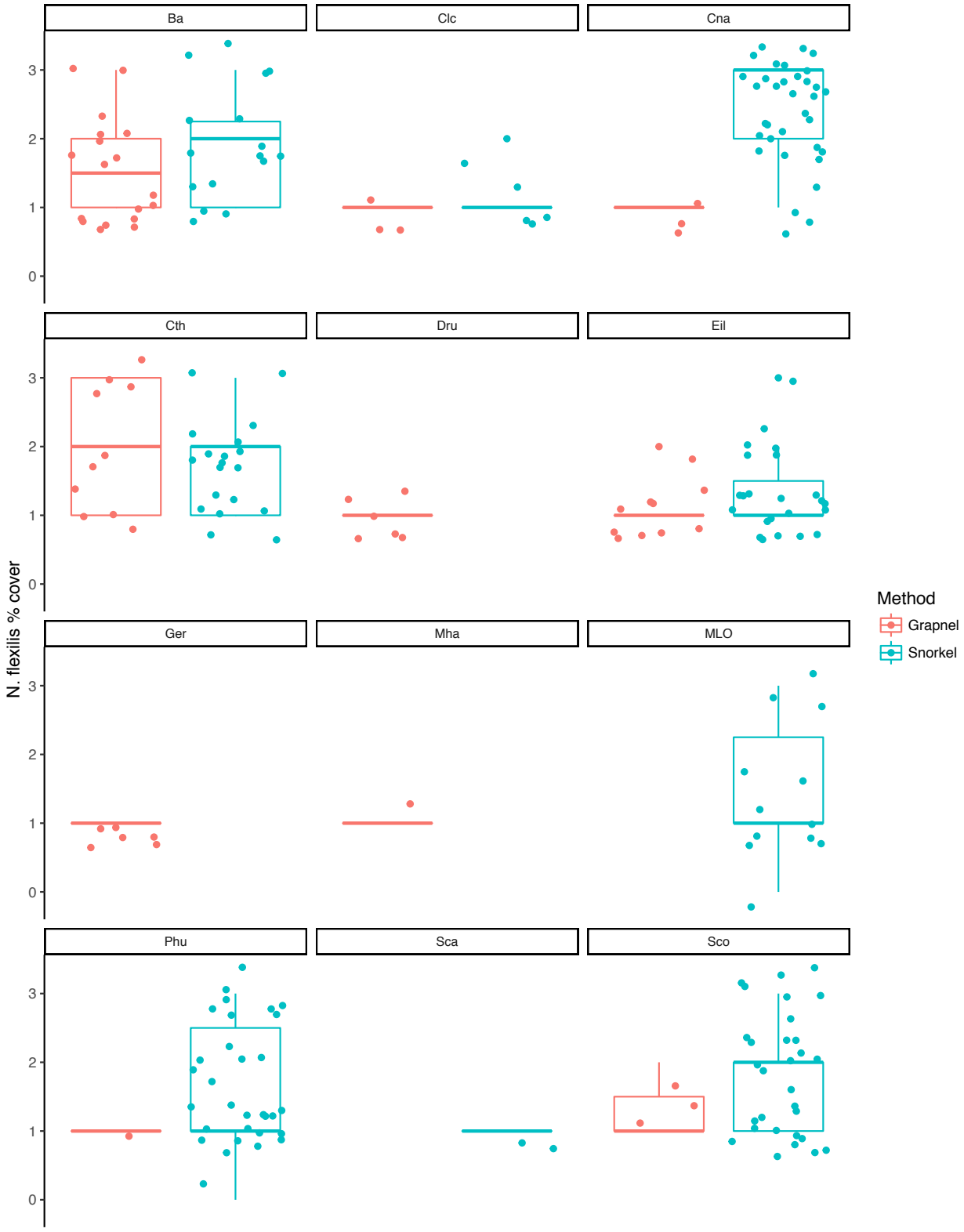

Figure 3.4 Boxplots comparing percentage cover of N. flexilis *recorded by grapnel (red) and snorkel (blue) during 2016 SCMs. Site name abbreviations are defined in table 2.1.*

3.2.2 Where is *N. flexilis* most likely to be found in a loch, based on variables that are readily available to surveyors?

Fig. 3.5 illustrates the variety of depth ranges at which *N. flexilis* was found to be growing across all sites, when results obtained by all methods were considered, arranged by median water depth. Across all sites, *N. flexilis* was found in a wide range of depths, from a minimum of 0.08m at Loch Tangy to a maximum of 2.2m at Glenastle Loch. The depth zones in which *N. flexilis* was found varied greatly between lochs, with some lochs exhibiting greater depth ranges than others. For example, at the shallow end of the spectrum, *N. flexilis* was only found between 0.75m and 1m at Loch Cuilc. At the deeper end, *N. flexilis* was found between 1.6m and 1.9m at Loch Scarie. *N. flexilis* was present at the largest range of depths (0.08 – 1.8m) at Loch Tangy. Fig. 3.6 shows how these depth ranges relate to the water chemistry variables measured at each site Kendall's Tau scores are shown in table 3.2, and show that there was no correlation between minimum, maximum and median depths of *N. flexilis* growth and the majority of variables measured. The exceptions were total dissolved solids and conductivity, which were positively correlated with minimum *N. flexilis* depth, pH, which was positively correlated with median *N. flexilis* depth, and Secchi depth, which was negatively correlated with maximum *N. flexilis* depth. Examination of Fig. 3.6 reveals that, with the exception of Secchi depth, these correlations may be influenced by single outlying datapoints at the highest end of the measured range.

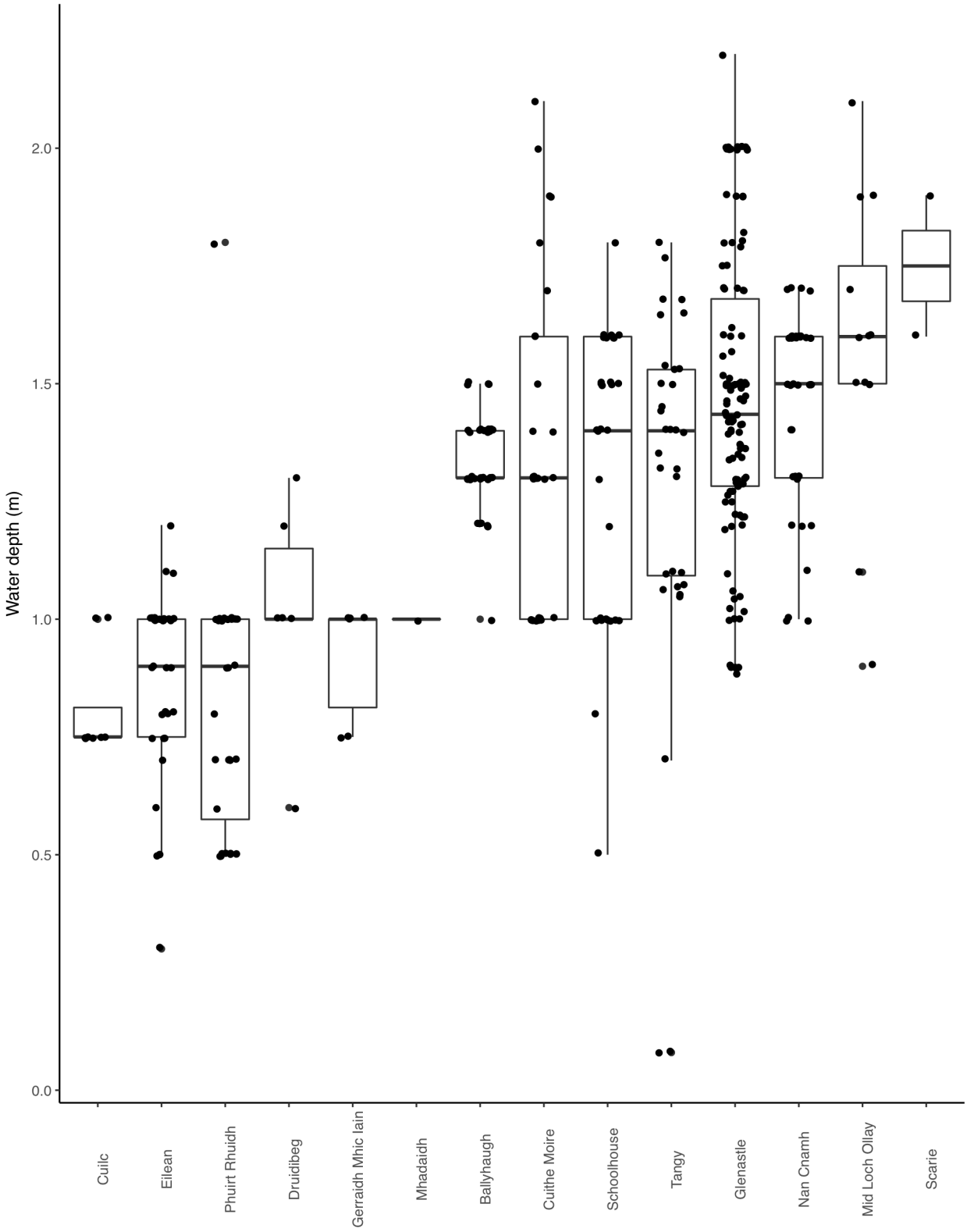

Figure 3.5 Range of depths at which N. flexilis *recorded during pilot studies and 2016 SCMs.*

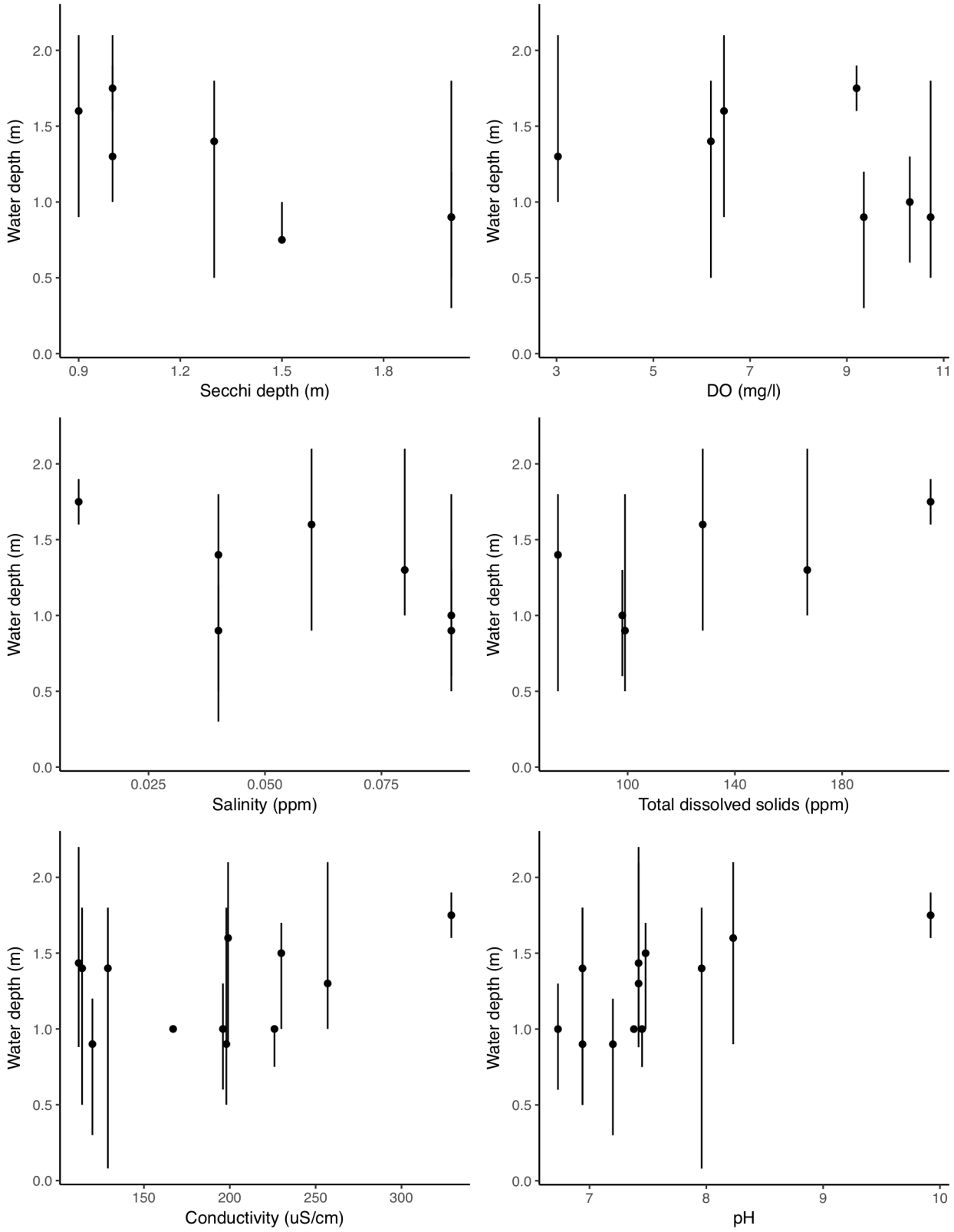

Figure 3.6 Depth range at which N. flexilis *found with relation to field measurements of water chemistry variables.*

Table 3.3 Results of tests for correlation between minimum, maximum and median water depth N. flexilis *and water chemistry measured in the field.*

	Min. *N. flexilis* depth	**Max. *N. flexilis* depth**	**Median *N. flexilis* depth**
Secchi depth (m)	T = -0.6156 P = 0.06	**T = -0.6842** **P = 0.04**	T = -0.5643 P = 0.09
Dissolved Oxygen (mg/l)	T = -0.2928 P = 0.36	T = -0.4506 P = 0.17	T = -0.2928 P = 0.36
Salinity (ppm)	T = -0.1026 P = 0.76	T = -0.0526 P = 0.88	T = -0.5130 P = 0.12
Total dissolved solids (ppm)	**T = 0.8281** **P = 0.02**	T = 0.5013 P = 0.17	T = 0.3333 P = 0.47
Conductivity (µS/cm)	**T = 0.5001** **P = 0.03**	T = 0.0473 P = 0.83	T = 0.1734 P = 0.44
pH	T = 0.3016 P = 0.19	T = 0.2241 P = 0.33	**T = 0.6242** **P = 0.01**

3.3 Discussion

3.3.1 Comparing the accuracy of grapnel surveys and SCUBA/snorkel surveys for *N. flexilis.*

Of the seven SCM sites which were surveyed using both snorkel and grapnel during the 2016 survey and at which *N. flexilis* was found, the plant was found at significantly deeper depths by snorkel than by grapnel at three sites and at shallower depths at one site

(Loch an Eilean). At another site – Loch Scarie – *N. flexilis* wasn't recorded by grapnel at all, despite being identified at two sample points in the snorkel survey. In most cases, this is likely because the grapnel surveys only extended to maximum wader depth and therefore did not cover the same depth range as the snorkel surveys. The exception is Loch an Eilean, where most *N. flexilis* was located within a shallow bay which was spanned in its entirety by wader surveys. At Loch Ballyhaugh, where boat transects were used (they were excluded from most sites on the basis that the lakes were too small, shallow and exposed for boat work to be beneficial), the number of times *N. flexilis* was recorded by grapnel was comparable to snorkel surveys. However, at Glenastle Loch and Loch Tangy, where grapnel surveys did extend to deeper water depths, they were found to under-represent *N. flexilis* populations. This suggests that, by design, the SCM surveys may not be adequate for the identification of *N. flexilis* populations without the addition of the supplementary identification of two specific *N. flexilis* populations, although, in the light of the results from Loch Ballyhaugh, further comparisons between boat transects and snorkel surveys are required to support this assertion.

The surveys from Glenastle Loch and Loch Tangy show that *N. flexilis* was consistently recorded more frequently using SCUBA than grapnel. The exception to this is between water depths of 1 – 1.5m at Glenastle Loch. This may be due to sampling bias; the distance between sample points on the SCUBA transects at this site was measured by fin kicks, and it is possible that this regular sampling strategy led caused a reduced number of points to be positioned in areas where the bed was sloping more steeply (i.e. the transition from shallow to deep water). This notwithstanding, the results from both Glenastle Loch and Tangy Loch suggest that *N. flexilis* is at particular risk of being missed by grapnels at water depths below approximately 1.5 – 1.6m. Furthermore, at Loch Ballyhaugh – one of the 2016 SCM sites – observations of *N. flexilis* made by snorkel came from significantly deeper water depths than those made by grapnel, even though the grapnel was thrown from a boat along a transect running from the deepest point in the lake to the shore. This finding is in line with the work of Capers (2000) on the same species in North America, where *N. flexilis* was present only as a deep-water plant. Wingfield *et al.* (2005) observed that *N. flexilis* is slender, fragile, difficult to uproot and does not float all the way to the surface. In contrast, grapnels tend to favour

robust, branching plants with high stem densities (Sheldon and Boylen, 1978). Since *N. flexilis* is slender, and is primarily a deep-water plant (Preston and Croft, 1997), methods that allow direct examination of the lake bed (i.e. snorkel and SCUBA surveys) are preferable to grapnel surveys, even in cases where the grapnel surveys cover the depth range of the entire photic zone.

Direct examination of the lake bed has further advantages over grapnel techniques that are less easily quantified. *N. flexilis* is a fragile plant and is known to fragment and float (Preston and Croft, 1997). Using a grapnel, it is not possible to distinguish between *N. flexilis* fragments that have been uprooted in situ and those that have floated in from elsewhere in the lake (James and Barclay, 1996). During the 2016 SCM snorkel surveys, it was noted that, at two of the sites, *N. flexilis* beds were forming a sub-canopy underneath a taller canopy of *Myriophyllum alterniflorum.* The physical structures formed by macrophytes in lakes are known to have important effects on faunal diversity and fish foraging, and, by providing habitats for invertebrates, can play an important structuring role in trophic interactions across the entire lake ecosystem (e.g. Wilcox and Meeker, 1992; Søndergaard and Moss, 1998; Valley and Bremigan, 2002). Although structural studies are beyond the scope of this PhD, it is important to note that such observations can only be made through direct examination of the lake bed using snorkel or SCUBA. At Glenastle Loch, SCUBA divers were able to observe a potential threat to *N. flexilis* that was not identified during grapnel surveys nor through water chemistry monitoring. Towards the western end of the loch, tower-like structures formed by some unidentified filamentous algae were covering *N. flexilis* plants (see Fig. 3.7). Such structures have the potential to prevent light penetration to macrophyte beds, as well as producing excess biomass that, when it decays, can lead to anoxia. Perhaps the most famous example of this is in the North American Great Lakes, where the filamentous algae *Cladophera glomerata* (Auer *et al.*, 2010). Significant abundances of filamentous algae were not recorded in association with *N. flexilis* at any other sites.

Figure 3.7 Photograph showing filamentous algae covering N. flexilis *at Glenastle Loch.*

3.3.2 Predicting the likely location of *N. flexilis* within a lake.

N. flexilis is known to grow in a wide range of water depths; it has been recorded growing at depths from 0.5m in Ireland (Roden, 2002) to 14m in Canada (Pip and Simmons, 1986). The results presented here provide further evidence for this tolerance of different water depths; the collective depth range for *N. flexilis* growth across all sites was 0.08m – 2.2m. However, when considering data from all twelve sites at which *N. flexilis* was present, there was a huge variety in the depth zone in which the plant was found at each site. A study by Wingfield *et al.* (2005) used TWINSPAN analysis of 52 UK lakes that either currently or formerly contained *N. flexilis* to show that *N. flexilis* is associated with different plant communities in different lakes, and that these associations are linked to pH, alkalinity, total organic nitrogen, soluble reactive phosphorus and light penetration.

In this study, a significant positive correlation was found between maximum *N. flexilis* depth and Secchi depth. This implies that *N. flexilis* is able to grow in conditions of low light penetration, and that, in some situations, at larger abundances than where light is less readily available. This is in contrast to most other macrophyte species, particularly deep water species like *Charophytes*, which are generally observed to grow in deeper waters in clearer lakes (Middelboe and Markager, 1997). However, since *N. flexilis* was found growing at a large range of different depths, including shallower depths in clearer waters, this does not appear to be a simple relationship and cannot be used to accurately predict the depth range at which *N. flexilis* is likely to be found. There is clearly scope for further investigation of the factors that influence the depth and location at which *N. flexilis* grows, and this will be addressed further in chapter 4. For the purposes of this discussion on survey methodology, it can be concluded that the identification of a fixed *N. flexilis* habitat zone within a lake based upon bathymetry and observable water chemistry variables is not possible. Any sampling strategy must therefore span the entire photic zone.

3.3.3 The practicalities of SCUBA/snorkel surveys.

The evidence presented here suggests that, since *N. flexilis* can be found at a range of water depths and grapnel methods are less accurate in deeper water, surveys should be undertaken either by SCUBA or snorkel and should cover as much of the lake bed as possible. However, there were a number of sites at which snorkel surveys were not possible due to cold conditions, diver illness, or other logistical considerations. There are numerous practical considerations to be made when designing survey methods: The survey must be cost-effective (i.e. affordable to the government agencies and contractors who typically undertake SCM surveys) , rapid enough that little time is spent on each individual waterbody, make use of equipment that is robust, mobile and easily repaired and require little time, effort and equipment to process samples (Nichols, 1984; Gunn, 2004). Wade and Bowles (1981) compared the cost, time taken and number of species recorded in shore-based, boat-based and snorkelling/SCUBA techniques. They found that, for deep water species, snorkelling/SCUBA based surveys recorded not only more species, but also took less time, than their boat-based counterparts. The findings of this

study concur with this. Table 3.3 summarises the relative merits of SCUBA, snorkel and grapnel methodologies observed during this study, in relation to surveys covering the entire area of a site. The surveys at Glenastle Loch and Tangy Loch were among the first to be carried out for *N. flexilis* using SCUBA, and there were numerous practical "teething problems". SCUBA had the advantage of allowing the surveyor to remain underwater always, meaning that plenty of time could be taken to identify plants and estimate percentage abundances. This, combined with trying to locate pre-assigned grid references, meant that the surveys were very time intensive. Any disturbance to the fine sediments by the divers obscured the view of the plants and made the survey impossible until the sediment had settled again. The divers had to "hover" just above the plants but below the surface of the water. In water depths of under 2m (most the photic zone), surface water tension acts to pull the diver back to the surface, and "hovering" required a great deal of very fine buoyancy control on the part of the diver. All dive equipment had to be transported to site, including air tanks and a portable compressor for air refills – these are cumbersome, and would be impossible to transport to remote sites. In contrast, snorkel surveys were time- and cost- efficient, technically simple enough to be carried out by anyone with strong swimming skills, and required minimal heavy equipment. They did, however, require more advanced macrophyte identification skills, because it was not possible to spend as long examining the plants in situ and identification of each species therefore had to be done quickly. It was also sometimes difficult to see the lakebed where visibility was reduced by either peat stained water, low natural light levels linked to the weather, or algal blooms. This meant that snorkelers sometimes had to dive under the surface in order to find *N. flexilis.* At Loch Scarie, a simultaneous snorkel survey undertaken by the RSPB failed to find *N. flexilis* because surveyors remained on the surface throughout (R. Watts, pers. comms). One disadvantage to "duckdiving" is that it risks disturbing sediments and damaging plants if not done with care, and, in an evaluation of the methods used during a survey of the Dunkeld-Bairgowirie Lochs for *N. flexilis,* James and Barclay (1996) favoured SCUBA for this reason. However, this was based on detailed surveys carried out at a single, mainland location. On balance, snorkel surveys including "duckdiving" seem to provide the most accurate survey results whilst remaining affordable and practical for surveys involving multiple sites in remote

locations.

One significant limitation of snorkel surveys is the fact that the field season is limited by the weather and risk of cold exposure. Grapnel surveys have the advantage of being possible throughout the growing season in all but the most inclement conditions. Whilst this study recommends snorkel surveys wherever possible, it is recognised that this is not always possible in situations where many sites must be visited by a small number of personnel over a single season. Boat transects, whilst often not used in smaller lakes, allow access to deeper areas of the site with a grapnel. These transects may still under-record *N. flexilis*, but do present a higher probability of capturing the full depth range of the species. Where snorkel surveys cannot be completed, the inclusion of boat transects is therefore important.

Table 3.4 Practical merits and disadvantages of SCUBA, snorkel and grapnel methods.

	SCUBA	**Snorkel**	**Grapnel**
Estimated equipment outlay and maintenance costs	Portable air compressor: £2000 Air cylinder: £200 (require two per person for a full day of survey work between evening refills) Bouyancy control device, weights, octopus and regulators (ballast and breathing equipment): £1000 per person Semi-dry suit, gloves, hood, boots and other specialist dive clothing: £500 per person Fins, mask and snorkel: £80 per	Semi-dry suit, gloves, hood, boots and other specialist dive clothing: £500 per person Fins, mask and snorkel: £80 per person Tow float (floatation device required for health and safety): £20 Safety boat and engine: £420 Quadrat: £12 Handheld GPS device: £75	Boat and engine: £420 Grapnel and bathyscope: £80 Chest waders: £30 per person Handheld GPS device: £75

	person Dive computer: £150 per person Safety boat and engine: £420 Handheld GPS device: £75 TOTAL (based on two divers): £6,775	TOTAL (based on one snorkeler): £1,107	TOTAL (based on two surveyors): £635
Personnel required	Two SCUBA divers and two non-divers in safety boat.	One snorkeller, and two non-swimmers in a safety boat or ashore.	Two field operatives.
Training required	Dive training by certified dive school (eg PADI qualifications), ideally including relevant first aid. Macrophyte ID. Basic field skills.	Confident swimmer. Macrophyte ID. Basic field skills.	Boat handling. Macrophyte ID. Basic field skills.
Estimated time taken	Two ~500m transects per day for two divers.	3-4 SCM sectors per day for one snorkeller.	3-4 SCM sectors per day.
Practical advantages	Can remain under water long enough to examine and identify plants in situ. Can detect things that are impossible to record using grapnel (e.g. coverings of filamentous algae).	The trained eye can identify plants in situ. Requires minimal equipment. Can detect things that are impossible to record using grapnel (e.g. coverings of filamentous algae).	Requires minimal equipment. Reduced risk of exposure to cold water.

Practical disadvantages	Difficult to navigate between sample points – often requires resurfacing. Requires extremely skilled buoyancy control to avoid disturbing sediment and obscuring view of plant bed with suspended silt. Cold exposure risk limits survey season and time spent in water. Requires bulky, technical equipment that is difficult to transport and repair.	Can be difficult to survey deeper waters, sometimes requires the snorkeller to "duckdive" below the water surface. Cannot get as close to plants as allowed by SCUBA. Requires physical fitness. Cold exposure risk limits survey season and time spent in water.	Impossible to directly observe the plant bed. Under-records *N. flexilis* at water depths > ~1.5m and where plant abundance is low.
Health and safety considerations.	Diving to depths >5m (unlikely during plant surveys in the UK) requires planning of length of dive and dive depth profile to avoid decompression sickness. Risk of cold exposure and hypothermia. Risks associated with underwater entanglement and drowning (low risk due to use of breathing apparatus and diver training). Defective equipment and contaminated air - mitigated by	Risk of cold exposure and hypothermia. Risks associated with underwater entanglement and drowning - snorkelers typically undergo less rigorous safety training than qualified SCUBA divers.	No special considerations outside those expected when conducting aquatic ecology fieldwork.

	following manufacturers instructions during air refills, and conducting thorough pre-dive checks.

New and developing technologies such as the use of hydroacoustics, underwater photography using autonomous underwater vehicles (AUVs), or remote sensing may offer alternative solutions to the problem of surveying *N. flexilis* in unfavourable weather conditions. In a study comparing the accuracy of grapnels, Ekman grabs, bathyscopes, hydroacoustics and underwater photography at Loch Leven, Scotland, Spears *et al.* (2009) found that hydroacoustics and underwater photography (conducted from a video camera attached to an extendable pole held over the side of a boat) provided the most accurate estimates of maximum colonisation depth, but that neither of these methods could successfully identify macrophytes to species level. AUVs offer the chance to position cameras closer to the lakebed to obtain clearer images. Most AUVs are too large to be used in the lacustrine environment, but there are some examples of their successful use, such as the AUV PURLII, used to record water temperature, conductivity and pressure at Loon Lake, Vancouver, Canada (Laval *et al.*, 2000). In the estuarine and coastal environment of Derwent Estuary, Tasmania, Davie *et al.* (2008) had some success classifying aquatic vegetation to species level by using a video camera mounted to an AUV and applying an algorithm to the spectral data from the images. Similar spectral analysis approaches have successfully been applied to remote sensing images of the Norfolk Broads to identify macrophytes to species level, including *Najas marina* (Hunter *et al.*, 2010). Remote sensing also offers the opportunity to assess canopy structures, and laser scanning approaches have already been applied to the terrestrial environment to this effect (Malhi *et al.*, 2018). However, the diffraction of light by the water column impedes such techniques in lakes, and the only successes to date have been in differentiating between floating, emergent and submerged plant stands (Hunter *et al.*, 2010). Compared to snorkel, grapnel and even SCUBA surveys, the acquisition of images by either AUV or

aerial/satellite photography is expensive, and the initial financial outlay required for such techniques needs to be carefully balanced against the practical advantage of being able to survey in difficult weather conditions as the technologies continue to develop.

3.3.4 Development of a new sampling protocol for *N. flexilis*.

During the course of this study, several different sampling strategies were tested. Although the transect-based sampling strategy used at Glenastle Loch was difficult to quantify, it had the advantage of providing a more complete qualitative description of the depth zone in which *N. flexilis* was growing, and the plants and physical characteristics attributed to it. This was achieved through the creation of sketches made on site, showing the floristic variation with depth observed whilst swimming each transect. An example of these sketches is shown in Fig. 3.8. The species associated with *N. flexilis* shown by these sketches are discussed in detail in chapter 4. In contrast, the point-based survey at Tangy Loch used sample points at a fixed geographic location in combination with a quadrat of a defined 1m2 area to provide a more quantitative estimate of *N. flexilis* abundance across the loch. In the past, several different approaches have been taken to snorkel survey designs with regards to sampling strategy. James and Barclay (1996) surveyed only the photic zones of the Dunkeld-Blairgowrie Lochs, working their way around the most easily accessed sections of the shoreline. This strategy excluded some potential *N. flexilis* habitats; one bay of Loch of Craiglush, for example, was not surveyed because the presence of floating macrophytes (e.g. *Nymphaea alba*) made snorkeling difficult. When the Dunkeld-Blairgowrie lochs were surveyed by snorkel in 2004 and again in 2007 (Murphy, 2007, unpublished; Benthic Solutions, 2007), it was established that most *N. flexilis* plants were growing at an approximate depth of 2m, and transects therefore followed the 2m isobath. The length of the transects were defined by the speed and efficiency of the snorkeler, making quantification of the abundance of the plant in each transect difficult (Benthic Solutions, 2007). The 2016 RSPB snorkel survey of Loch Scarie (R. Watts, pers. comms) addressed this by swimming transects from one shore of the loch to the other, similar to the approach used in this study at Glenastle Loch, but this is impossible and inefficient in larger, deeper lochs.

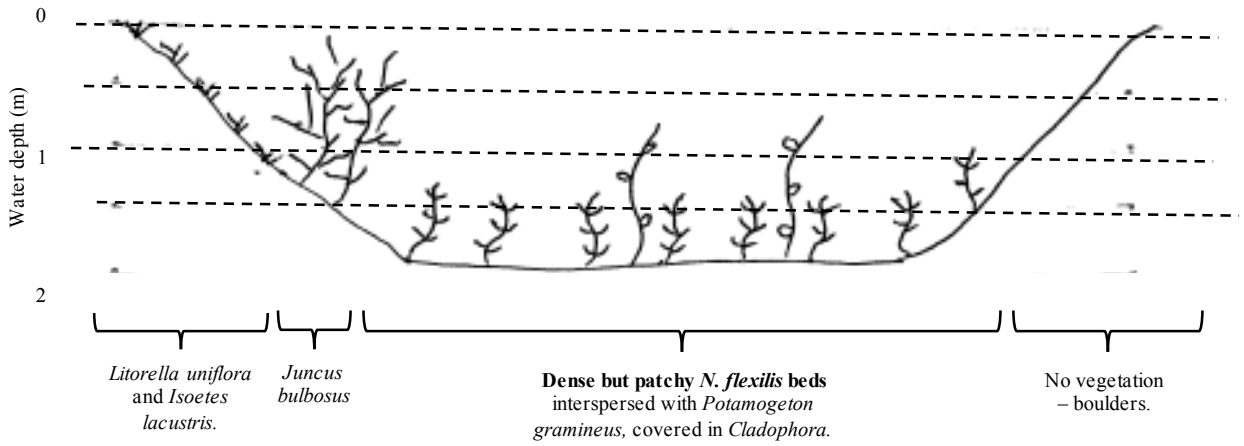

Figure 3.8 Example of transect sketch from Upper Glenastle Loch. All transects can be found in Appendix 2.

During the 2016 SCM surveys, a snorkeling survey protocol was designed to fit around existing SCM surveys. The protocol incorporated the positive aspects of both transect and point-based sampling approaches, as well as drawing on the practical lessons learned during this study. The protocol was found to be cost-effective, time efficient and to provide sufficient quantitative data for subsequent statistical analysis. The full survey protocol is described in Fig. 3.10, and it is recommended that this methodology is applied to future *N. flexilis* surveys. The method fulfills the SCM requirement to record species composition of 20 quadrats within two populations of *N. flexilis*, but additionally clarifies that these 20 quadrats should all lie between the minimum and maximum depths of observed *N. flexilis* colonisation at the site rather than encompassing the entire habitat-depth zonation within which *N. flexilis* is growing. This clarification ensures that data collected across all sites reflects the abundance of *N. flexilis* within its potential habitat, and eliminates bias against sites with steeply shelving beds with a smaller area of potential *N. flexilis* growth. Transect depth profile sketches were found to provide valuable information on the variation in location of the *N. flexilis* zone in relation to other plants both at Glenastle Loch and the sites surveyed in 2016, and are used in the recommended protocol to provide context to the quadrat data. Another key difference between the existing SCM methodology and the recommended protocol is the fact that the snorkel transects are in fixed locations, based around the existing SCM transects. In

the 2016 survey, the sampling strategy based upon SCM transects led to the identification of two populations of *N. flexilis* at all sites at which the plant was present except for Loch Scarie, where *N. flexilis* was rare and additional sampling effort was required to find the plant. This approach means that, rather than assessing *N. flexilis* populations in different locations every survey year, surveys are recording the success of *N. flexilis* within a habitat that is consistent and comparable across different years (as far as this is possible within a dynamic lake environment). Changes noted in the frequency, abundance and colonisation depth of *N. flexilis* and associated species over time can therefore be more reliably attributed to environmental change rather than biases introduced by changes in survey effort, location, or methodology. Furthermore, in cases where *N. flexilis* has disappeared, the same habitat can be consistently assessed to determine which species have replaced the plant. In this case, the twenty quadrats would be thrown within the last known depth range of *N. flexilis*.

Starting at the beginning of the 100m SCM sector, a snorkeler should swim out from the shoreline to the maximum depth of vegetation colonization (or to the opposite shoreline if the entire lake basin is vegetated), and return back to the shore approximately 20m further along the sector, creating zig-zag pattern similar to that shown in Fig. 3.10. It may be necessary to swim underwater for some of this distance if the secchi depth is less than the depth of maximum vegetation colonization. The aim of this swim is to assess the general habitat and the way in which *N. flexilis* features within the vegetation zonation. At the end of this swim, the snorkeler should be able to make a qualitative sketch of the habitat similar to the example given in Fig. 3.11. The important features to note are approximate depths of major vegetation changes, changes in sediment type and approximate locations of *N. flexilis* stands in relation to distance along the sector/water depth. If *N. flexilis* is found, the minimum and maximum depths of *N. flexilis* should be recorded. Within this depth range plus 0.5m water depth on either side to allow for future changes, and covering the entire 100m sector length, 20 quadrats should be thrown at random and the water depth, sediment type and percentage cover of all plants present in the quadrat should be recorded. The easiest way to do this is to have the snorkeler shout out the observations to a scribe who is standing on the shore. To save time, it can be done at the same time as the zig zag swim. The population of *N. flexilis* can later be assessed quantitatively by using the number of quadrats out of twenty in which *N. flexilis* was present/abundant in combination with the size of the depth range occupied by the plant. If no *N. flexilis* is found, the survey should be extended outside of the original transects until two populations have been found. If *N. flexilis* is still not found, the 20 quadrats should be thrown in the same location as the last known population of *N. flexilis,* within the same depth range as the last known record.

Continued overleaf.

Figure 3.9 Recommended protocol for snorkel surveys of N. flexilis

Box 3.10 cont

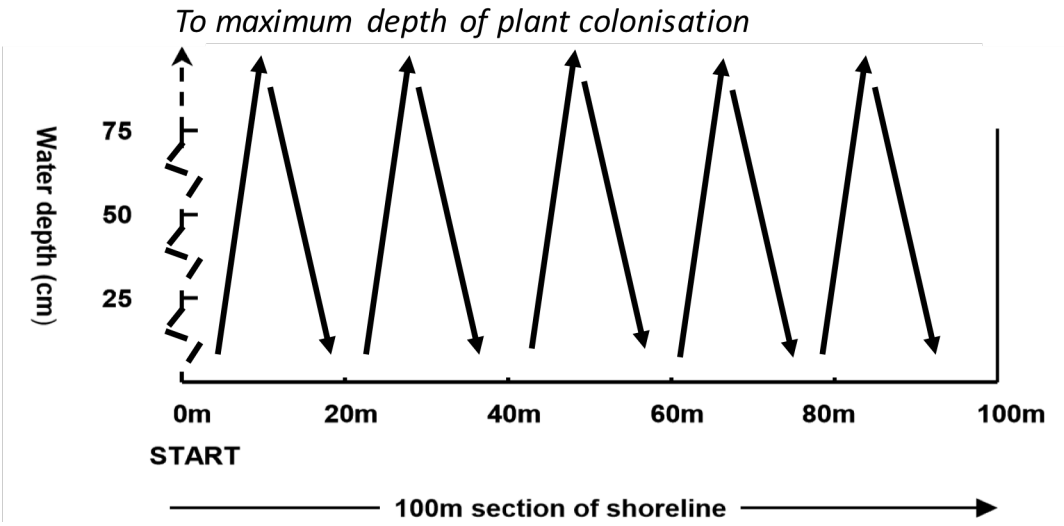

Figure 3.10 Recommended zig-zag pattern for snorkel surveys of SCM sectors.

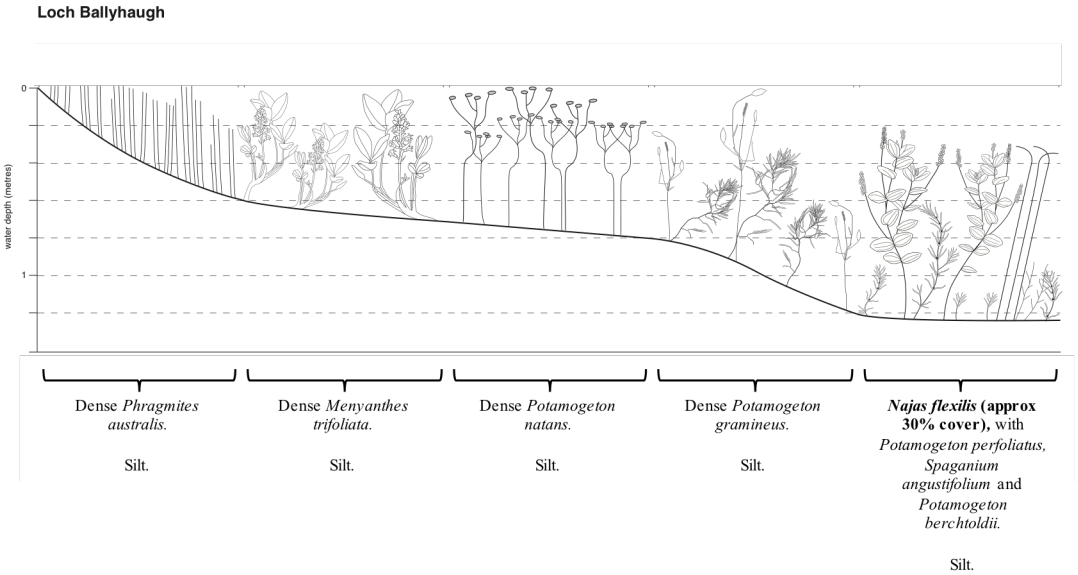

Figure 3.11 Example of a transect depth-zonation sketch, from Loch Ballyhaugh.

3.3.5 Future applications of snorkel surveys.

With the development of the use of environmental DNA (eDNA) to detect the presence of target species, the effectiveness of traditional survey methods is under new and increased scrutiny, particularly in the aquatic environment. Within the space of a few hours, eDNA from species that live in any standing-water aquatic habitat can be extracted from a water sample and identified (Rees *et al.*, 2014). eDNA detection is not only quick, it is also accurate. In water samples taken from mesocosms containing known numbers of six rare European aquatic animals, detection rates ranged between 80-100% for all species, both in terms of positive detection when the species were present and negative detection two weeks following removal (Thomsen *et al.*, 2012). Such techniques have already been tested for the application of rare species monitoring in the UK; In 2013, volunteers collected eDNA samples from 239 ponds across the UK from known Great Crested Newt (*Triturus cristatus*) sites in order to test both the efficacy of the method and the ease with which large-scale surveys could be conducted by citizen scientists (Biggs *et al.*, 2014). Tests of the effectiveness of eDNA at detecting the presence of *N. flexilis* are also currently underway (N. Crutchley, pers. comms.). However, eDNA techniques can produce false-positives and false-negatives, and are currently unable to provide accurate information on the abundance or exact location of the target species (Rees *et al.*, 2014). In the case of *N. flexilis,* the combination of eDNA to identify potential *N. flexilis* sites with snorkel surveys to confirm the presence, abundance and location of the species has potential to drastically improve upon current monitoring strategies.

3.4 Conclusions

The evidence presented here suggests that grapnel surveys under-record *N. flexilis* compared to in-water surveys, particularly at water depths below ~1.5m. Although SCUBA surveys are expensive, take considerable time and require equipment that is not easy to transport, snorkel surveys provide the option to use in-water techniques in a cost- and time- effective manner. Despite being more effective, snorkel surveys are more practically challenging than grapnel surveys, and this should be taken into consideration when planning field campaigns. However, the practicalities do not completely preclude

the use of snorkel surveys, and they should be adopted wherever possible. The results of past surveys that do not use such methods should be interpreted with caution. Since chapter 4 uses the results of past surveys to investigate the habitat preferences of *N. flexilis,* care has been taken to ensure that the results of snorkel surveys are included in the analyses wherever possible. *N. flexilis* grows in different depth zones at different sites, therefore it is not possible to establish the likely zone of *N. flexilis* growth based upon variables easily measured on site. It is therefore necessary to survey the entire photic zone to guarantee finding *N. flexilis;* it is not possible to use water chemistry to inform a stratified sampling strategy. A snorkel-based sampling strategy based around existing SCM protocols is recommended. This strategy is based around a fixed geographical location that is consistent in time, ensuring that surveys are comparable across time. Furthermore, by consistently surveying in the same place, *N. flexilis* populations can be examined with relation to a relatively fixed morphological habitat, and any changes through time can be more accurately attributed to environmental changes. This protocol could easily be applied to other rare deep-water aquatic species, and multiple target species could be assessed in this way in a single survey. In combination with newly developed eDNA techniques, this snorkeling protocol has the potential to quickly assess the presence and abundance of *N. flexilis* at new sites as well as improving existing monitoring strategies.

4. The Ecology of *N. flexilis*

4.1 Introduction

This chapter uses existing SCM data alongside new data to investigate the types of environments that *N. flexilis* grows in, with an emphasis on identifying the causes of recent changes in *N. flexilis* distribution in Scotland. It asks the following questions:

1. What is the current status of *N. flexilis* in the British Isles and in Scotland in particular?
2. Under what conditions does *N. flexilis* currently grow in Scotland?
3. Does *N. flexilis* occupy different habitats in different types of lakes?
4. How directly do *Elodea* species compete with *N. flexilis* within the same habitat?

To assess the current status of *N. flexilis* (defined here as the status of the plant based on surveys undertaken within the last ten years), a database containing all existing *N. flexilis* records for lakes in the British Isles was created. This database was updated using results of SCM surveys since 2000 and the results of the surveys of Glenastle Loch, Tangy Loch and Loch of Butterstone conducted as part of this PhD research in 2013 and 2014. The sites contained within the database were then split into three groups based upon the results of surveys conducted during the last ten years: a) sites where *N. flexilis* was present during the last survey; b) sites where *N. flexilis* was absent during the last survey; c) sites which have not been surveyed within the last ten years and the current status of *N. flexilis* is unknown.

The results of SCM surveys conducted since 2000 was taken as representative of "current conditions". This definition was deemed more appropriate than taking the results of only the last survey because of the annual nature of *N. flexilis* and the high likelihood of inter-annual variations. The differences in water chemistry and vegetative community compositions between sites where *N. flexilis* was present and those where *N. flexilis* was absent were compared. Because of the detailed reporting of results from individual sample points required by SCM, comparisons of the micro-habitat (water depth,

substrate, exposure and % cover *Elodea* spp.) of sample points containing *N. flexilis* and those not containing *N. flexilis* was also possible. Given indications that *N. flexilis* may grow in different vegetative communities in different lakes as suggested by Wingfield *et al.* (2006), along with observations of *N. flexilis* occupying different depth and vegetative zones during the snorkel surveys (chapter 3), TWINSPAN analysis of SCM results was used to further investigate this assertion. This analysis allowed observations to be made regarding likely threats to *N. flexilis* occurrence in different lake types. These predictions are presented in this chapter, and, in chapter 6, are explored further from the long-term perspective offered by paleoecology.

4.2 Results

4.2.1 What is the current status of *N. flexilis* in the British Isles, and in Scotland in particular?

To date, *N. flexilis* has been found at a total of 126 sites in the British Isles since it was first recorded by Daniel Oliver in Cregduff Lough, Galway, Ireland, in 1850 (Oliver, 1851) – 62 in Ireland, 60 in Scotland and three in England. The apparent expansion of *N. flexilis* in the British Isles since 1850 is likely a result of increased survey effort rather than a genuine increase in the distribution of the plant. Indeed, this is reflected in Fig. 4.1, which shows an increase in the number of sites at which *N. flexilis* was recorded and a decrease in the number of sites at which its status was unknown as the century progresses. Of the 126 sites at which *N. flexilis* has been recorded since 1850, 82 have been surveyed for macrophytes in the last ten years (i.e. since 2007); the current status of *N. flexilis* is therefore unknown for 26 sites in Ireland, 14 in Scotland and one in England. Since 2007, *N. flexilis* has been found at 48 sites in the British Isles. There are currently 32 former *N. flexilis* sites at which the plant can no longer be found; 15 in Ireland, 15 in Scotland and two in England. *N. flexilis* disappeared from two English sites in the 1980s (with *N. flexilis* not recorded since 1883, and therefore assumed lost, at the third), and from 11 of its 17 locales on the Scottish mainland during the 2000s. More recent losses

have occurred in the Western Isles of Scotland, including from Loch Grogary (North Uist) in 2010.

Of the 59 current and former *N. flexilis* sites in Scotland, 26 are subject to regular SCM monitoring and have been surveyed at least once since 2000. Fig. 4.2 (overleaf) shows the abundance of *N. flexilis* at each of these sites during each survey as well as results from the surveys from this study at Glenastle Loch and Loch of Butterstone in 2013 and Tangy Loch in 2014. *N. flexilis* was absent from all sites on the Scottish mainland in all survey years, apart from Tangy Loch. Where *N. flexilis* was present, its abundance varied from <10% of sample points (e.g. Loch a'Mhaddaidh in 2016) to being present in almost 75% (e.g. Loch Ballyhaugh in 2004). Some sites (e.g. Loch Fada) had a consistent coverage of *N. flexilis* across years, whilst others (e.g. Loch Druidibeg) showed considerable variation in the percentage of sample points containing *N. flexilis* across years. The only site at which *N. flexilis* was lost during this period was Loch Grogary, where it was observed in 17% of sample points in 2010 but was found to be absent in an extensive snorkel survey in 2016. *N. flexilis* also showed a marked decline at Mid Loch Ollay, from 40% of sample points in 2010 to 9% of sample points in 2016. *N. flexilis* appeared to be absent from Loch Scaraidh in 2010, but was found in 2016 following an extensive snorkel survey. Another snorkel survey of the site carried out on the same day in 2016 by a different research team failed to find *N. flexilis* (RSPB, pers. comm.), and it is unclear whether *N. flexilis* was in fact present in 2010 but not found.

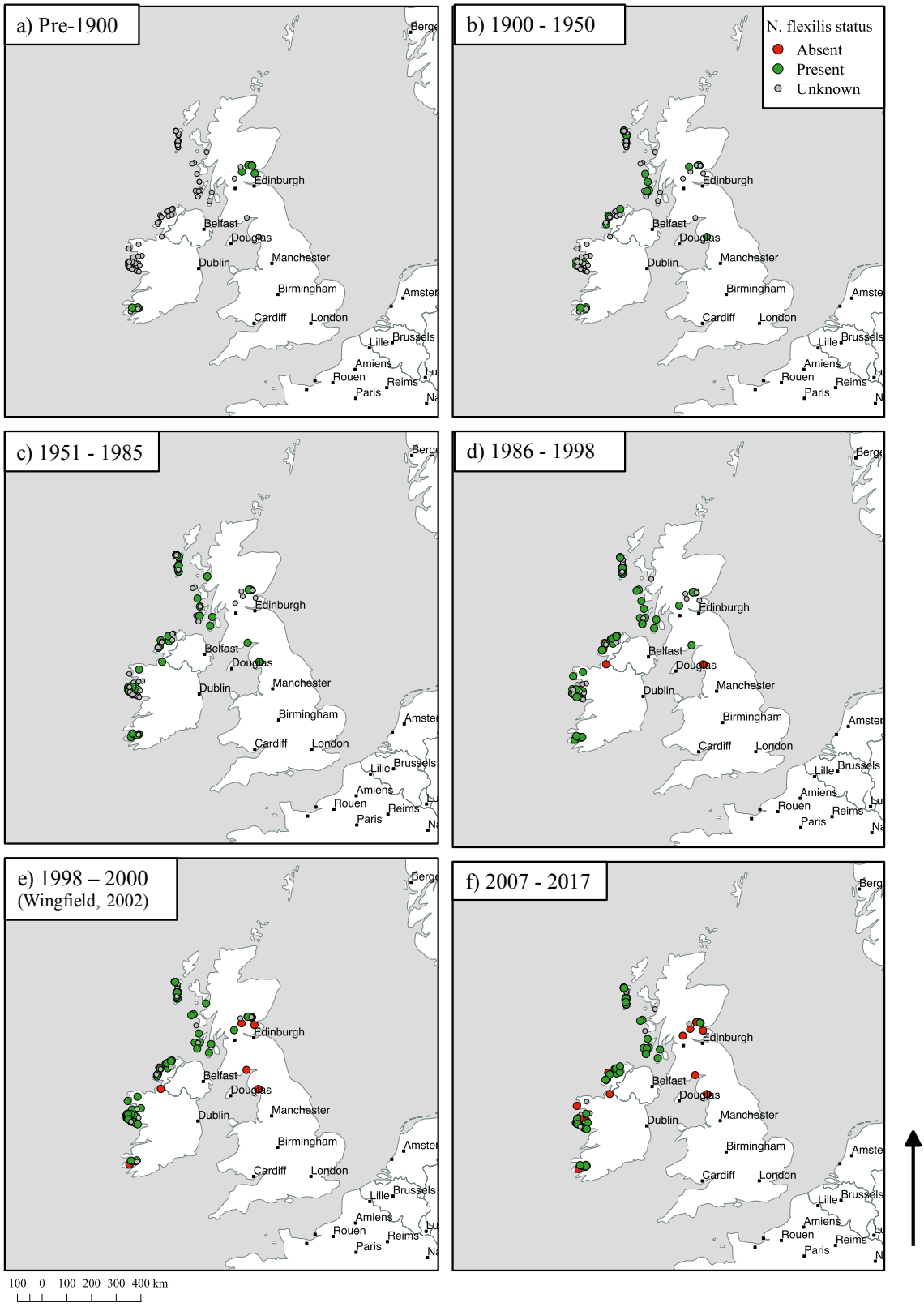

Figure 4.1 Status of N. flexilis *at all sites for which records exist a) before 1900, b) between 1900 and 1950, c) between 1951 and 1985, d) between 1985 and 1998, e) during*

the surveys of Wingfield (2002), and f) from 2007 to present. "Present" (green) represents sites with positive records for N. flexilis*, "absent" (red) represents sites that were surveyed but* N. flexilis *was not found, and "unknown" (grey) represents sites that were not surveyed, or where survey results are not available.*

4.2.2 In what conditions does *N. flexilis* currently grow in Scotland?

Lake-wide conditions

N. flexilis was found at a wide range of sites in terms of measured alkalinity, conductivity, pH and light extinction coefficients (LECs), but all sites at both former and current *N. flexilis* sites had low nutrient concentrations; nitrate concentrations were below 0.13 mg/l and total P concentrations were below 0.03 mg/l. Scores for statistical tests of difference between means of the group where *N. flexilis* was present and the group where *N. flexilis* was absent are shown in Table 4.1. Mean pH was significantly lower at sites where *N. flexilis* was found (7.4 where *N. flexilis* was present compared to 7.9 where it was absent), whilst mean nitrate concentration, although low at all sites, was significantly higher where *N. flexilis* was present (0.08 mg/l compared to 0.01 mg/l). In Kendall's Tau tests (table 4.2), significant positive correlations were found between *N. flexilis* site percentage cover and both LEC and nitrate concentration, and a significant negative correlation was found between *N. flexilis* site percentage cover and pH. All significant relationships are shown in Fig. 4.3. The sites included in each group, alongside the water chemistry data analysed, are listed in appendix 3.

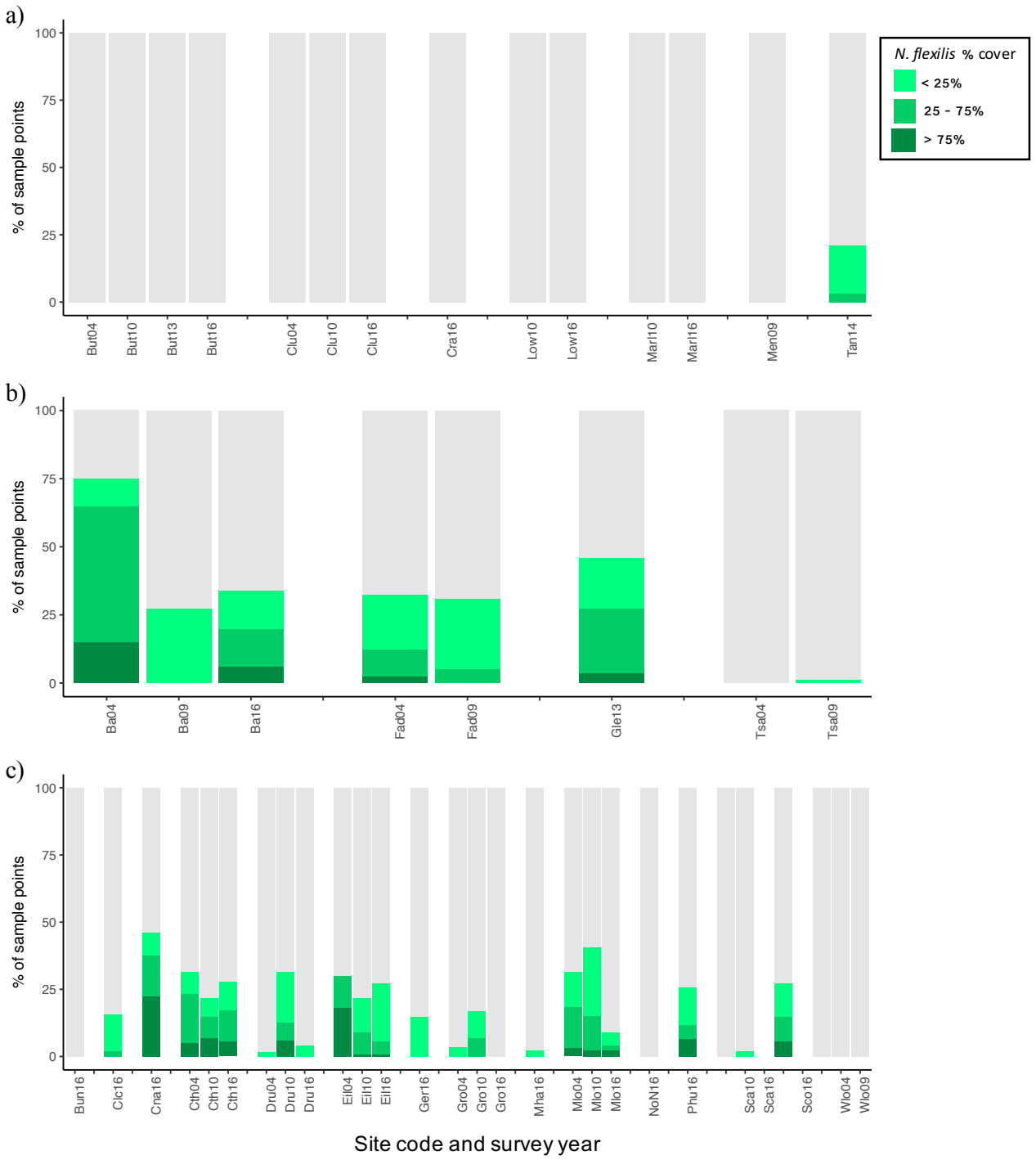

Figure 4.2 Figure 4.4.3 Percentage of sample points during each SCM survey in which N. flexilis *percentage cover was <25% (light green), 25 - 75% (mid green) and >75% (dark green). a) Mainland Scotland, b) Inner Hebrides, c) Western Isles of Scotland. Site name abbreviations are defined in table 2.1.*

Table 4.1 Results of tests for differences of mean water chemistry between sites at which N. flexilis *was present and sites at which it was absent.*

Variable	Test used	Test value	P value
Alkalinity as $CaCO_3$	Mann-Whitney	W = 32	0.5859
Conductivity	Mann-Whitney	W = 311	0.1876
LEC	Mann-Whitney	W = 240	0.0601
Nitrate	**Mann-Whitney**	**W = 113**	**0.0003**
pH	**Mann-Whitney**	**W = 124**	**0.0028**
Total P	T-test	t = -1.4129	0.1782

Table 4.2 Results of tests for correlation between site water chemistry variables and percentage of sample points containing N. flexilis.

Variable	T value	P value
Alkalinity as $CaCO_3$	-0.0429	0.8136
Conductivity	0.1527	0.1585
Dissolved oxygen	-0.2722	0.2344
LEC	**0.2653**	**0.0285**
Nitrate	**0.6442**	**0.0002**
pH	**-0.2837**	**0.0084**
Salinity	0.3420	0.1400
Total dissolved solids	0.000	1.000
Total P	-0.2842	0.1326

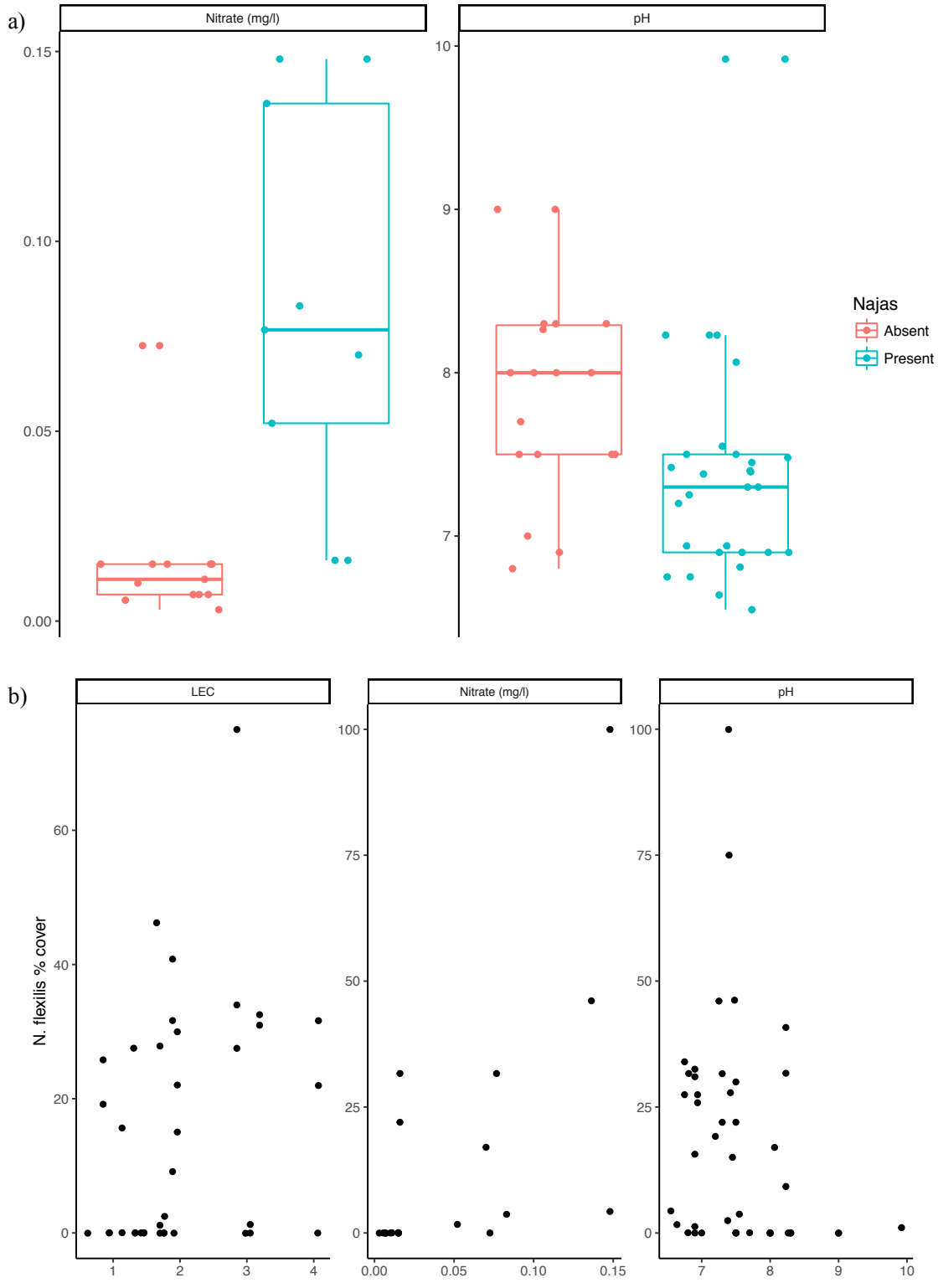

Figure 4.3 Relationships between significant water chemistry variables and a) N. flexilis *presence/absence and b)* N. flexilis *abundance.*

In a Principal Components Analysis of the total species composition of each site survey (Fig. 4.4), 62% of the variation was explained by the first four axes. The first two axes explained 43% of the variation. The full list of species and the abbreviations used can be found in appendix 4. Many species, including the invasive *Elodea canadensis,* are clustered towards the centre of the plot, indicative of the occurrence of these species more commonly at most or all sites. The variation between sites is explained by a few rarer species lying further from the centre of the plot, including *N. flexilis.* Species that were found in sites associated with *N. flexilis* include *Sparganium erectum, Potamogeton polygonifolius, Potamogeton lucens, Potamogeton crispus* and *Chara virgata. N. flexilis* sites were less likely to contain *Lobelia dortmana, Lemna minor, Lythrum portula* and the invasive species *Elodea nuttallii.*

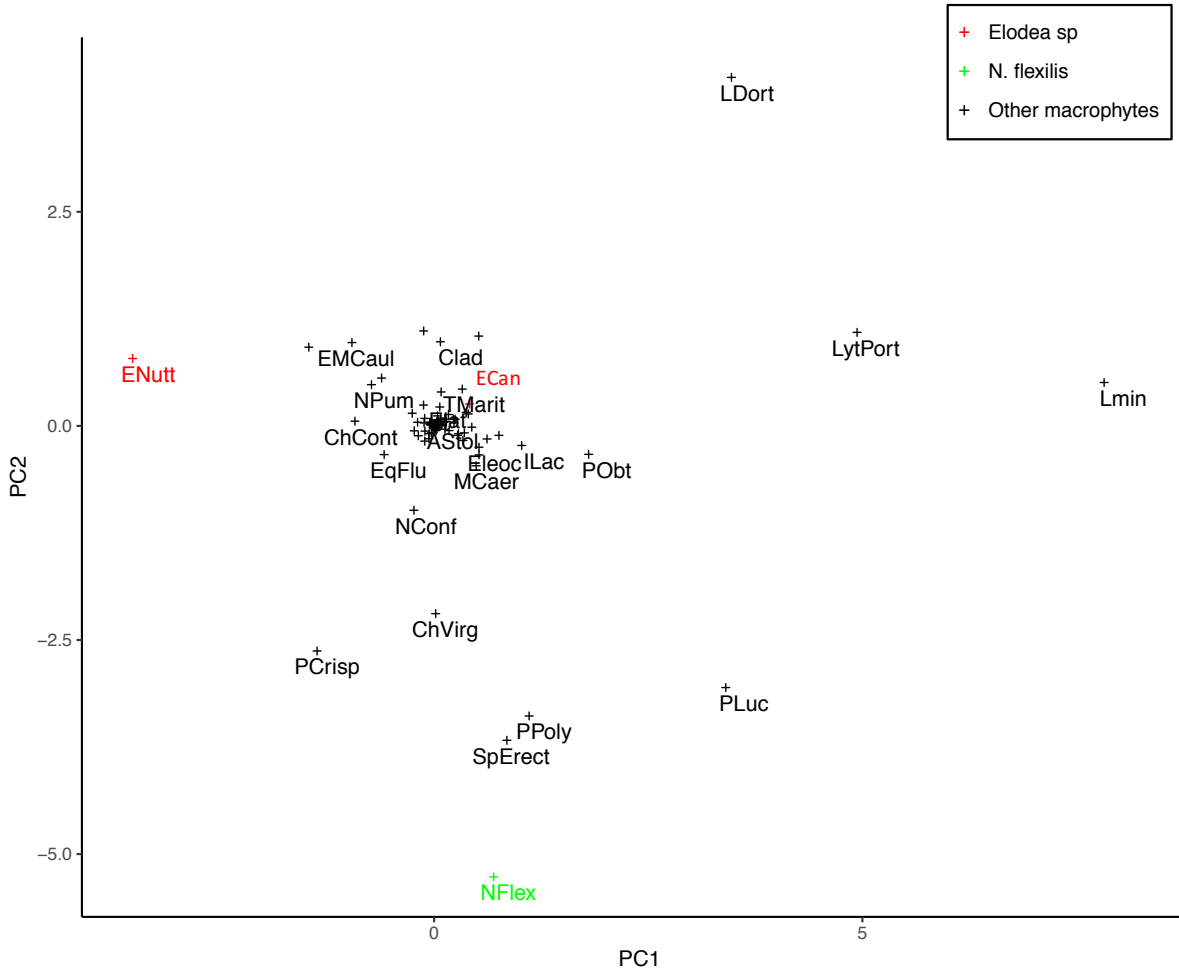

Figure 4.4 Principal Components Analysis of macrophyte percentage cover at each SCM site. Plotted axes explain 43% of variation. Abbreviations are defined in Appendix 4.

Micro-habitat conditions - water depth and exposure

In total, 4168 individual data points were analysed. Six hundred and fourteen (15%) of these sample points contained *N. flexilis.* Points at which *N. flexilis* was present were generally found in areas of lower wind exposure and greater water depths than points without *N. flexilis;* these differences were statistically significant in Mann-Whitney tests (exposure W = 683580, $P < 2.2 \times 10^{-16}$; depth W = 358410, $P < 2.2 \times 10^{-16}$). Furthermore, there were significant differences between the water depths at which *N. flexilis* percentage cover was <25%, 25 – 75% and >75%, with *N. flexilis* found at higher coverage values in deeper water (Table 4.3). No significant differences were found between mean *N. flexilis* percentage cover and exposure, but a significant positive correlation was evident between the two variables, with *N. flexilis* found at greater water depths where exposure was high ($\tau = 0.3460$, $P < 2.2 \times 10^{-6}$) (Fig. 4.5).

Table 4.3 Results of tests for differences of means of exposure and water depth at sample points with different N. flexilis *percentage cover.*

	Exposure		**Water depth**	
	25 – 75%	**>75%**	**25-75%**	**>75%**
<25%	W = 8384.5 P = 0.26	W = 4933 P = 0.21	**W = 21915** **P = 0.01**	**W = 8873.5** **P = 1.51×10^{-5}**
25-75%		W = 3404.5 P = 0.54		**W = 7668** **P = 0.03**

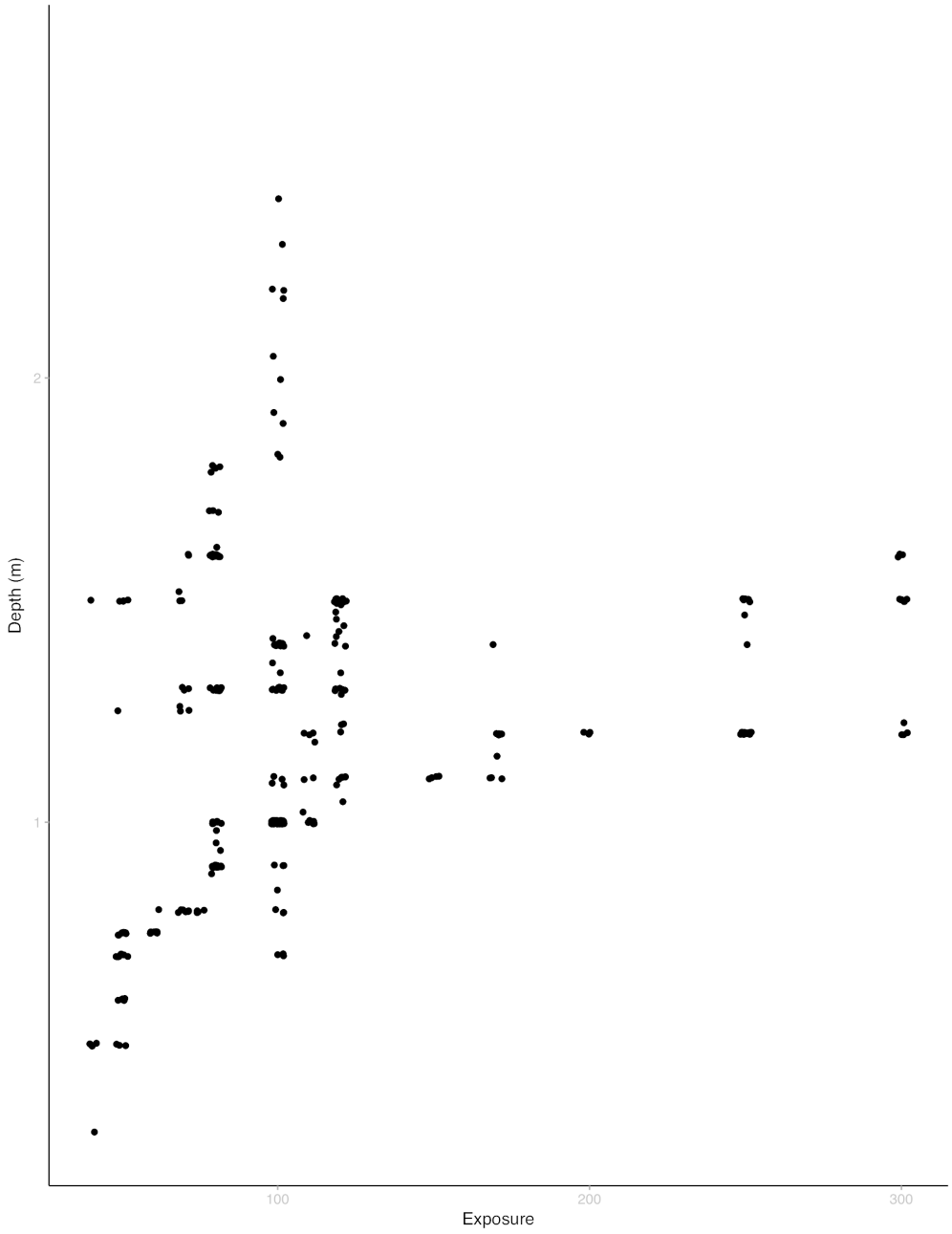

Figure 4.5 Relationship between depth and exposure for sample points at which N. flexilis *was found.*

Micro-habitat conditions - sediment characteristics

Fig. 4.6 shows the main substrate types associated with *N. flexilis.* Sample points at which *N. flexilis* percentage cover was >75% lay exclusively on sand and silt, although *N. flexilis* was found at lower abundances on boulders, cobbles, pebbles, gravel, sand, and

silt. When sample points for which substrate type was unknown were excluded, a Pearson's chi-squared test for association showed a significant difference between substrate types at points where *N. flexilis* was present and points where it was absent (X^2 = 432.6, P = 2.2 x 10^{-16}), with *N. flexilis* most strongly associated with silt (r = 14.903). *N. flexilis* abundance was also found to be significantly associated with substrate type (X2 = 20.52, P = 0.02), with sample points with a *N. flexilis* percentage cover > 75% the most strongly associated with silt (r = 0.860).

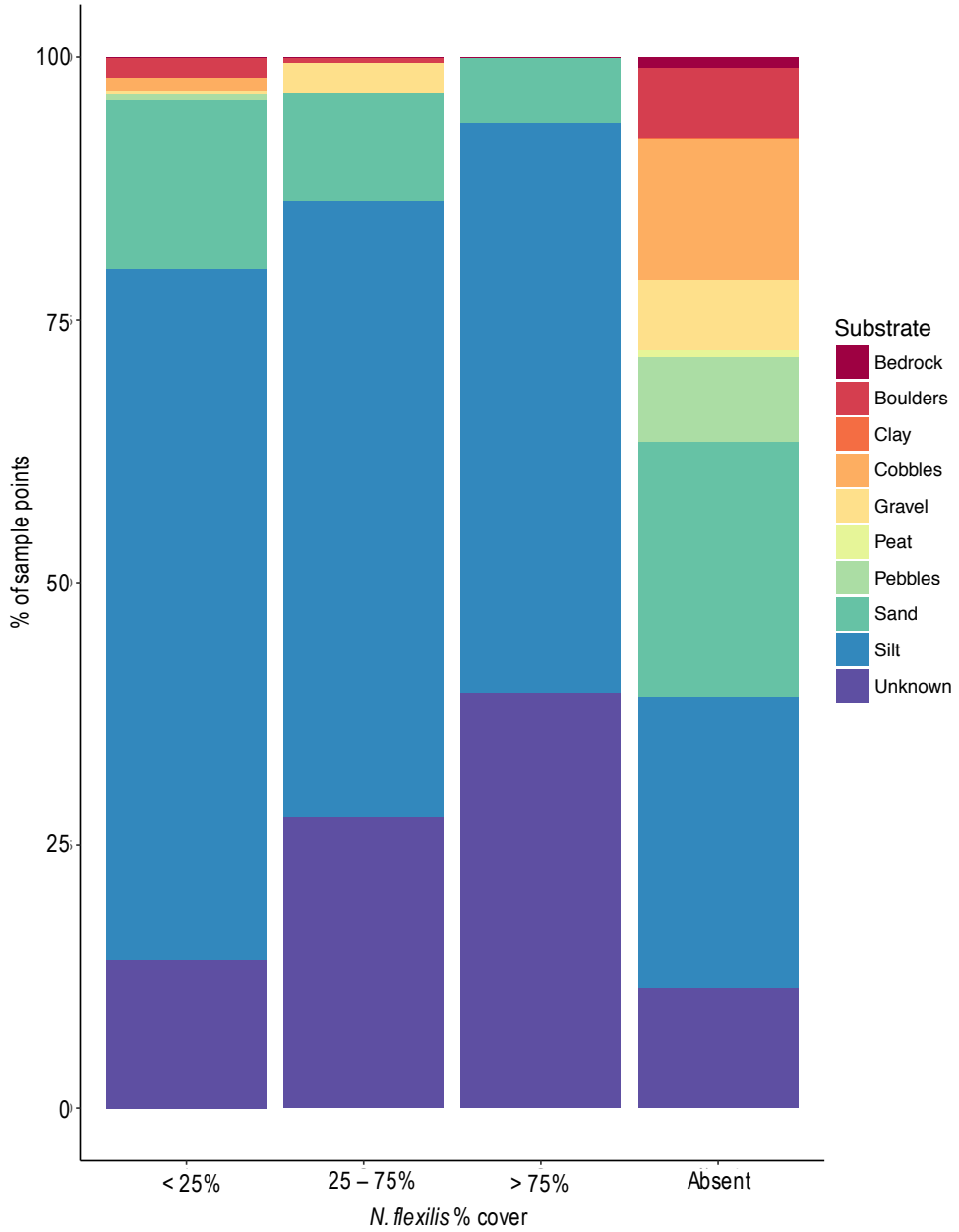

Figure 4.6 Sediment type at sample points of differing N. flexilis abundance

At Glenastle Loch and Loch

Tangy, sediment shear stress was measured in addition to sediment type. The range of measured shear stress was greater at Glenastle Loch than at Loch Tangy by close to one order of magnitude, with sediments at Loch Tangy generally much more fluid (Fig. 4.7). *N. flexilis* was also found in sediments with a significantly higher shear stress at Glenastle Loch than at Loch Tangy ($W = 1810$, $P = 5.28 \times 10^{-13}$). At Upper Glenastle Loch, a significant positive non-linear correlation was found between *N. flexilis* percentage cover and sediment shear stress (τ - 0.34, $P = 9.22 \times 10^{-9}$), whilst, at Tangy loch, a significant negative correlation was found between the same variables once one outlier had been removed ($\tau = -0.19$, $P = 0.04$) (Fig. 4.6).

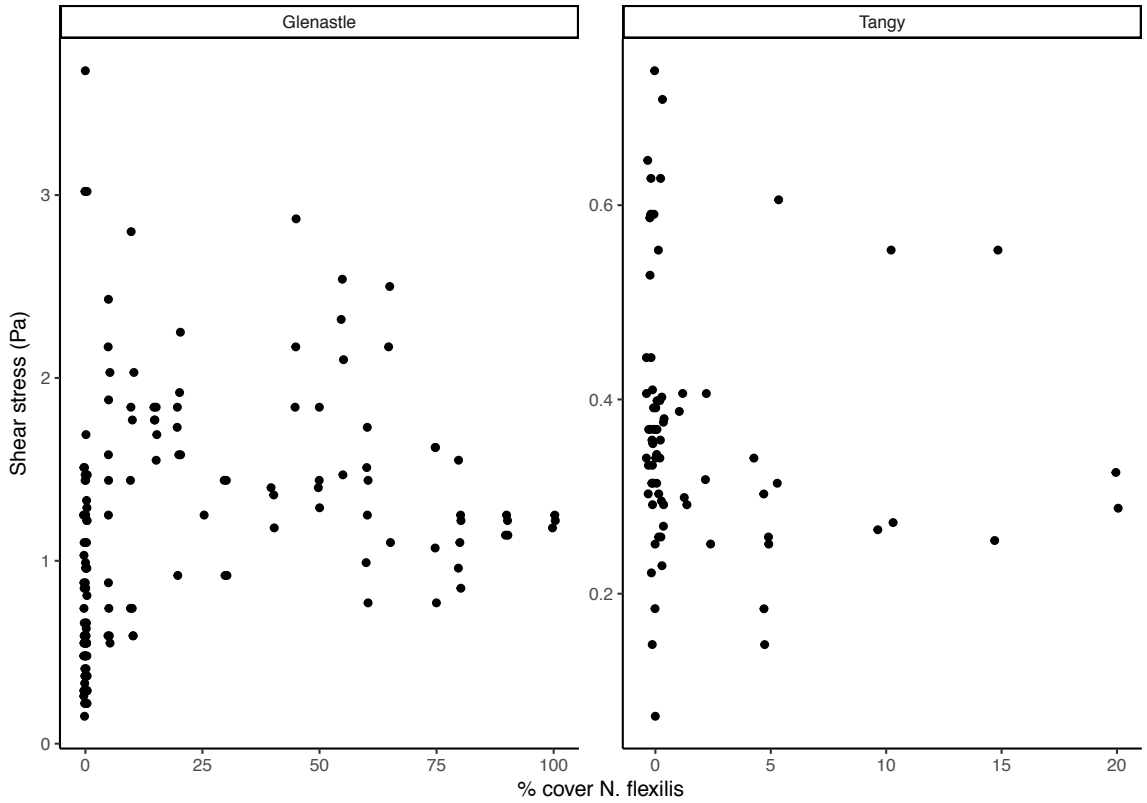

Figure 4.7 Relationship between N. flexilis percentage cover and sediment shear stress at Glenastle Loch and Loch Tangy.

Micro-habitat conditions - associated species

By treating each individual sample point from the SCMs separately, rather than grouping them by site (as in Fig 4.4), it was possible to assess which species were associated with *N. flexilis* at the micro-habitat scale. The first four axes of a PCA explained 40% of the variance of the species composition between individual sample points, with axes 1 and 2 together explaining 26% of the variance (Fig. 4.8, overleaf). Most species were clustered together in the centre of the plot, indicating that many species were present in most sample points. *N. flexilis* is located away from this cluster. Samples containing *N. flexilis* were more closely associated with the occurrence of *Potamogeton perfoliatus, Potamogeton gramineus* and *Potamogeton berchtoldii,* but no very strong associations between *N. flexilis* and other macrophyte species were evident.

4.2.3 Does *N. flexilis* occupy different vegetative zones in different types of lakes?

The transect sketches made during the snorkel surveys suggest that *N. flexilis* grows in different types of habitats in different lakes. Four different types of transect sketches were encountered: a) *N. flexilis* growing deeper than all other plants, either as sparse, occasional plants or in dense, mono-specific stand; b) *N flexilis* growing within a narrow depth zone, usually between a shallower species rich zone and a deeper zone of fine-leaved *Potamogeton* spp.*;* c) *N. flexilis* growing throughout the photic zone; d) *Elodea canadensis* occupying expected zone of *N. flexilis* growth. Examples of each of these transect types are shown in Fig 4.9, while all transect sketches are collated in Appendix 5.

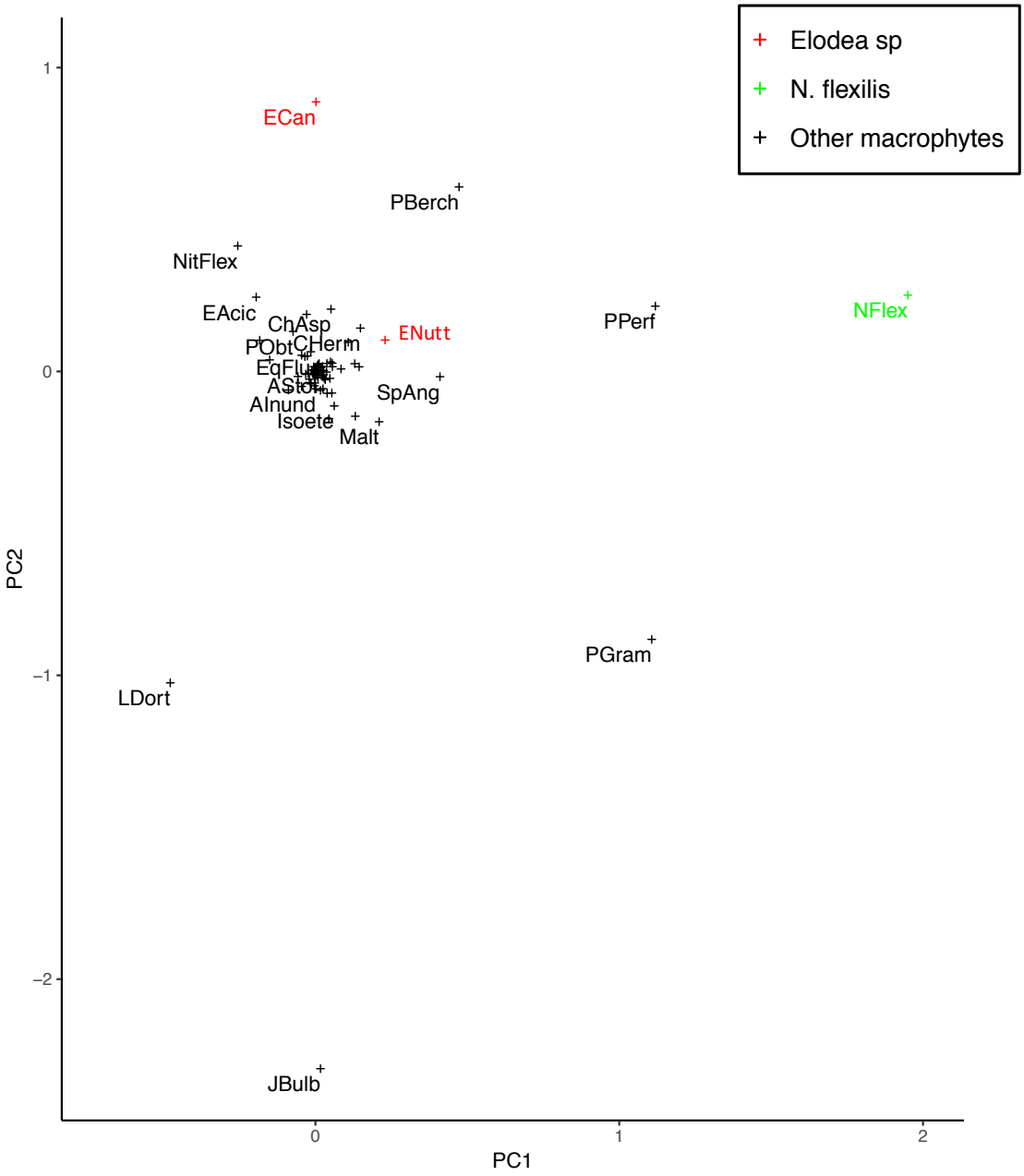

Figure 4.8 Principal Components Analysis of macrophyte percentage cover at each individual sample point within all SCMs. N. flexilis *is highlighted in green and* Elodea *species in red. Plotted axes explain 26% of variance. Abbreviations are defined in Appendix 4.*

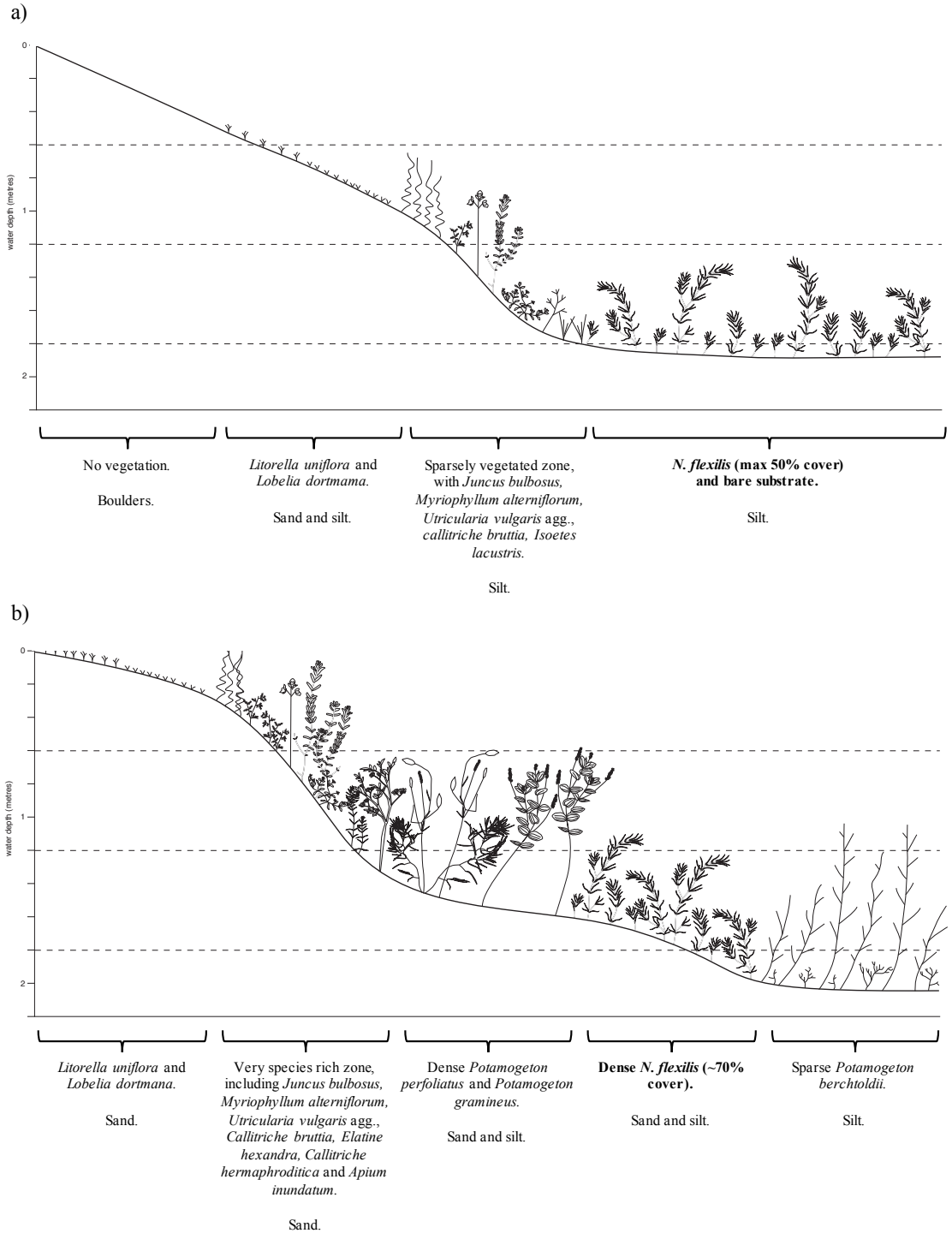

Figure 4.9 (continued overleaf).

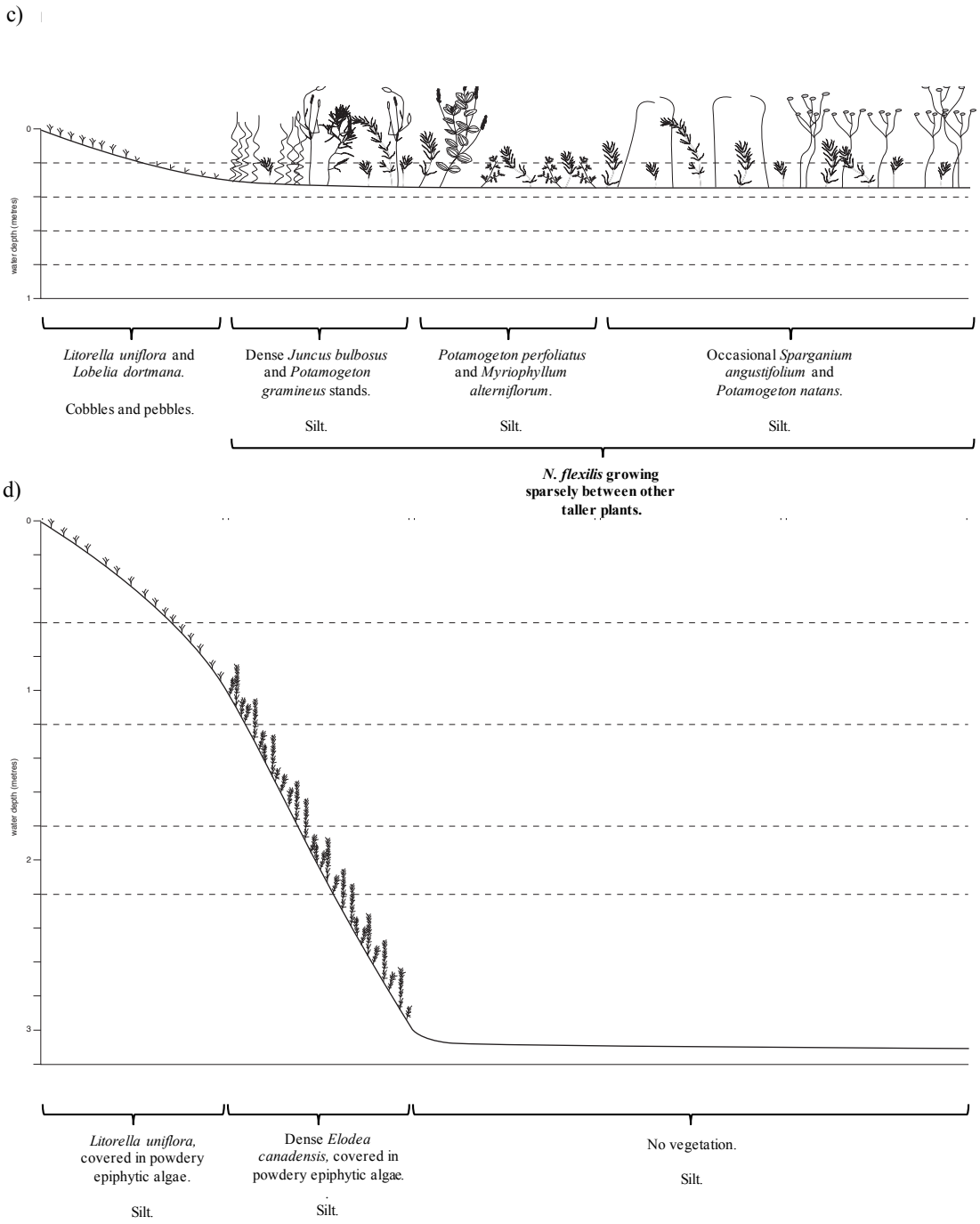

Figure 4.9 Snorkel transect sketches showing a) N. flexilis *growing deeper than other plants (Loch an Eilean); b)* N. flexilis *growing in a narrow depth zone (Loch a Cuithe Moire); c)* N. flexilis *growing throughout the photic zone (Loch Cuilc); d)* Elodea canadensis *occupying expected zone of* N. flexilis *growth (Loch of Craiglush).*

TWINSPAN analysis of the site species composition recorded in each SCM survey allowed further exploration of the differences in *N. flexilis* habitats across different sites. TWINSPAN revealed 10 groups (a to j) based upon their species composition. These groups are described in table 4.4. When plotted in a PCA, the main divisions in TWINSPAN are between groups A, B and G, groups C, D and E, groups F and J, and groups H and I (Fig. 4.10). Sites from across Scotland are split between these groups, suggesting that floristic variation isn't solely due to biogeographical factors. Henceforth, groups will be discussed in terms of the four main "divisions" (1 to 4) identified by combining the TWINSPAN output with the PCA, with the 10 groups referred to as "subgroups" (a to j). The clustering heirachy of these divisions and subgroups, along with key indicator species, is shown in Fig. 4.11.

N. flexilis was found in three of the four main site divisions, and thus with different communities of plants (Fig. 4.12). The highest proportion of individual sample points containing *N. flexilis* were found at sites in division 3 (subgroups f and j; 44% and 46% respectively), whilst division 4 contained very few individual sample points with *N. flexilis* (only 6% of sample points at subgroup H sites, and no sample points in subgroup I). All subgroups in division 2 contained *N. flexilis* at 10 – 25% of sample points. Division 1 did not contain *N. flexilis*.

Table 4.4 SCM surveys (site code + survey year) grouped according to species composition using TWINSPAN. Site codes are defined in chapter 2.

Division	1			**3**	
Subgroup	a	b	g	**f**	**j**
Sites	Mainland Scotland Butt4 Butt10 Butt13 Butt16 Lowe10 Lowe16 Marl10 Marl16	Mainland Scotland Craig16	Mainland Scotland Clun4 Clun10 Clun16	Mainland Scotland Tang13 Tang14 Inner Isles Bally4 Fada4 Fada9	Inner Isles Glen13

Division	**2**			**4**	
Subgroup	**c**	**d**	**e**	**h**	i
Sites	Inner Isles Bally9 Bally16 Tsag4 Tsag9 Western Isles Cuilc16 Mhad16 NoName16	Western Isles Bun16 Cnamh16 Cuithe16 Gerr16 Phui16 School16	Mainland Scotland Ment9 Western Isles Cuithe4 Cuithe10 Druid4 Druid10 Druid16 Eilean4 Eilean10 Eilean16 MLO4 MLO10 MLO16	Western Isles Grog4 Grog10 Grog16 Scar16	Western Isles Sca10 WLO4 WLO10 WLO16

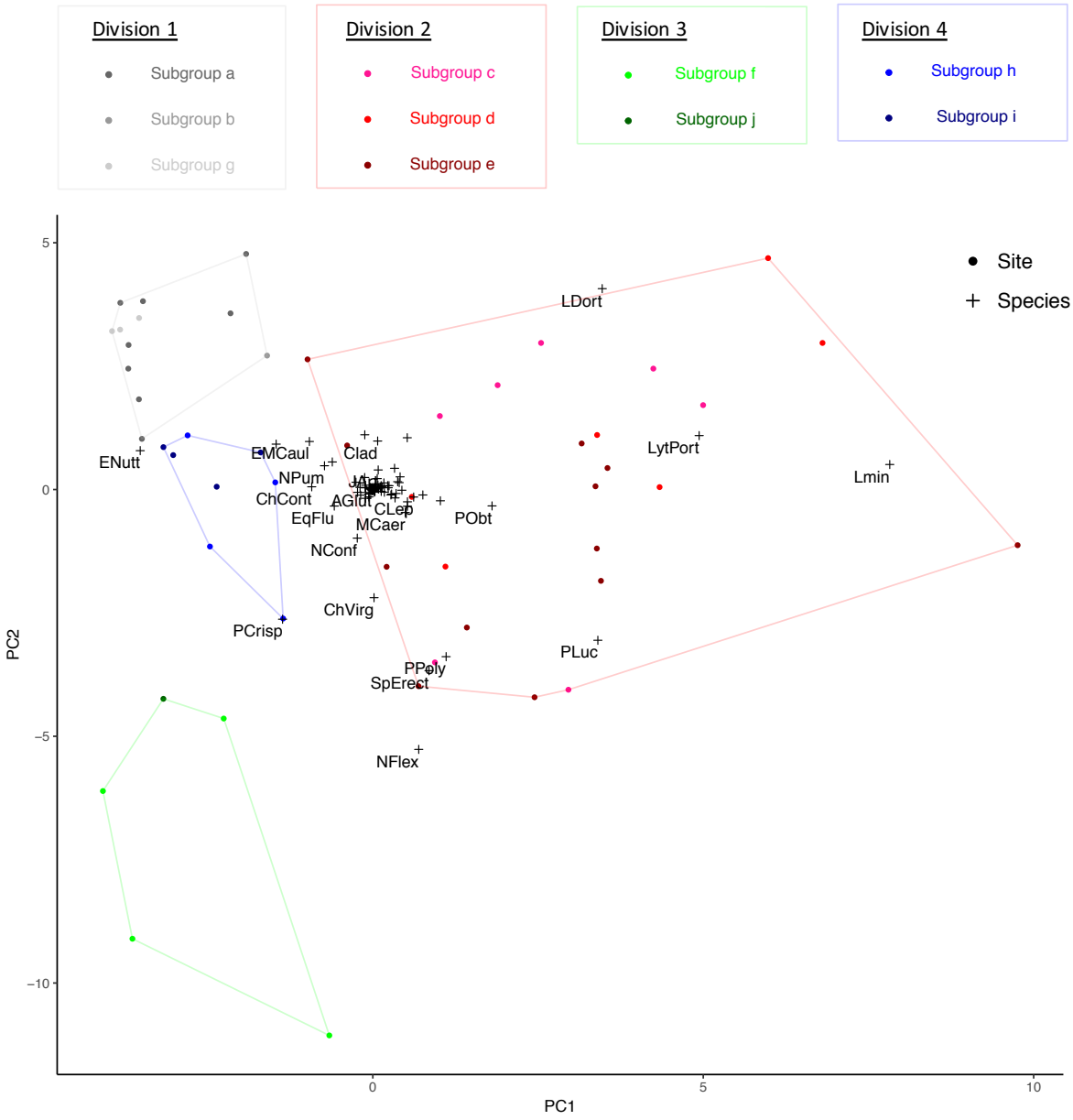

Figure 4.9 Principal Components Analysis of macrophyte percentage cover at each SCM site, showing sites grouped according to results of TWINSPAN analysis. Abbreviations are defined in Appendix 4.

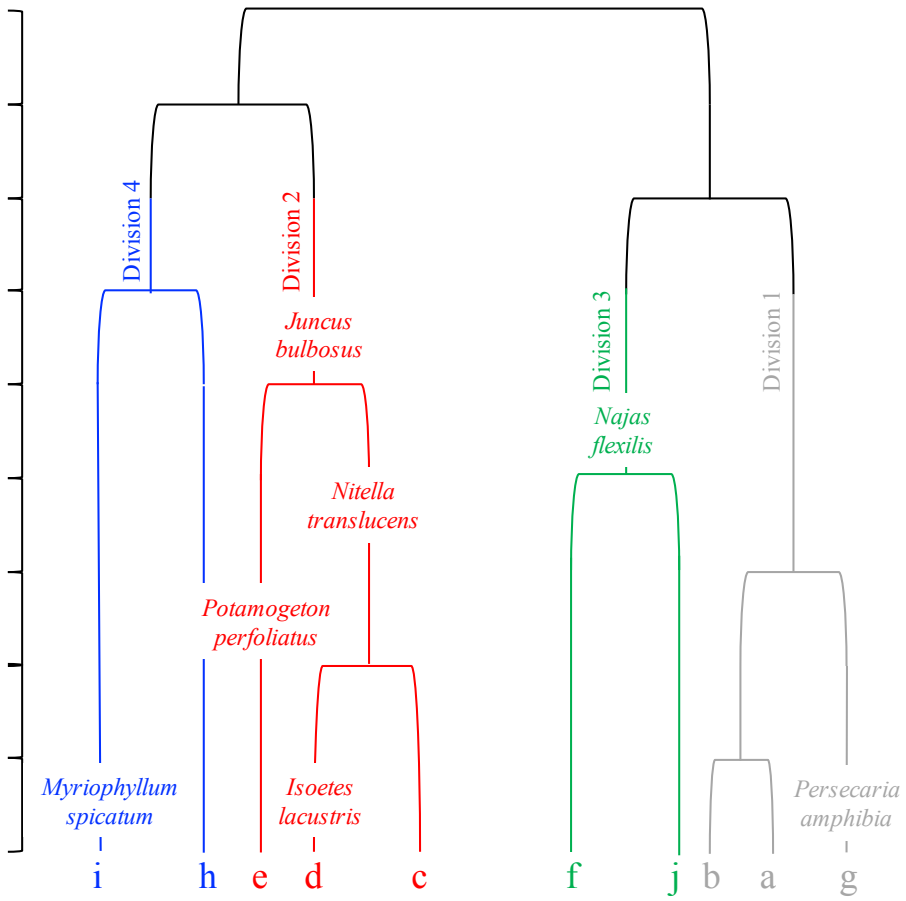

Figure 4.11 Dendrogram showing TWINSPAN groupings, with each division highlighted by colour. Key indicator species are shown in italics.

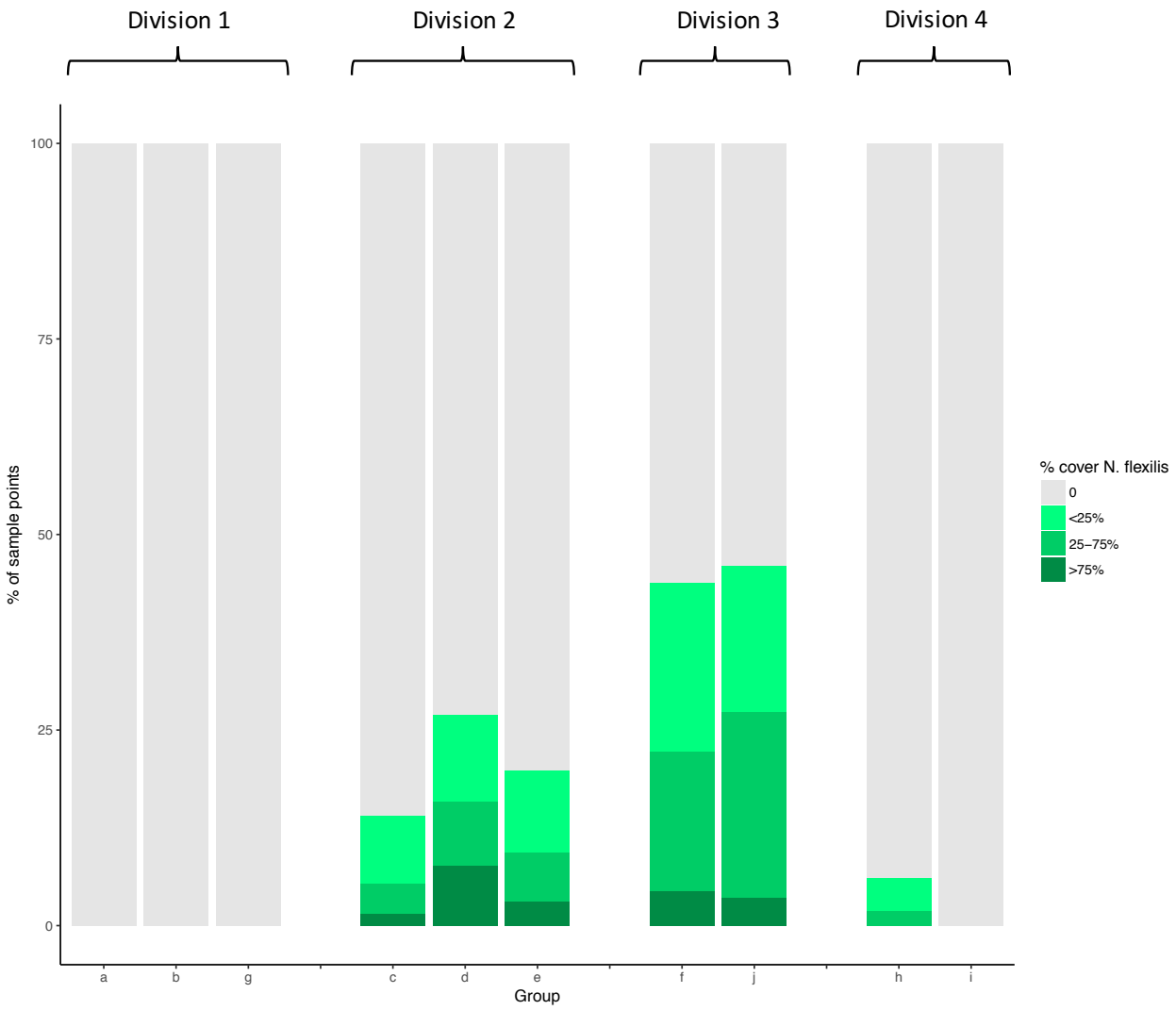

Figure 4.10 Percentage of sample points in each TWINSPAN group in which N. flexilis percentage cover was <25% (light green), 25 - 75% (mid green) and >75% (dark green).

Table 4.5 shows the species composition of each subgroup. The indicator species for division 1 was *Eleocharis acicularis,* with other species including *Elodea canadensis, Isoetes lacustris* and *Nitella flexilis* agg.. Indicators of division 2 sites were *Juncus bulbosus, Nitella translucens, Potamogeton perfoliatus, Isoetes lacustris* and *Apium inundatem*. These sites were particularly species rich. Correspondence analyses of the species composition of individual sample points (Fig 4.13) showed that, in this division, *N. flexilis* grew in species rich sample points, often associated with *Callitriche* spp. In subgroup C (Fig. 4.13a), *N. flexilis* was closely associated with *Callitriche bruttia var.*

hamulata, Potamogeton berchtoldii, Chara spp and *Isoetes echinospera*. In subgroup D (Fig. 4.13b) it was associated with *Callitriche hermaphroditica*, *Callitriche bruttia, Potamogeton perfoliatus* and *Apium inundatem.* Subgroup E (Fig. 4.13c) was the most species rich group, containing a total of 83 different aquatic plant species. In this group, species associated with *N. flexilis* included *Utricularia* spp., *Myriophyllum* spp., *Callitriche hermaphroditica, Isoetes* spp., *Elatine hexandra, Potamogeton berchtoldii, Potamogeton praelongus* and *Potamogeton pectinatus*. *N. flexilis* was an indicator species for Division 3, which also contained high abundances of *Potamogeton berchtoldii* and *Potamogeton perfoliatus* (Fig. 4.14). In subgroup F (Fig. 4.14a), *N. flexilis* was associated with *Callitriche platycarpa, Myriophyllum spicatum* and *Isoetes lacustris,* and in subgroup J (Fig.4.14b), it was associated with *Potamogeton praelongus, Nitella flexilis* agg, and *Nitella translucens*. The indicator species for Division 4 sites were *Myriophyllum spicatum* and *Elodea nuttallii.* These sites contained a diverse Charophyte flora. *Chara aspera* was present in high abundances, but *Chara virgata, Chara hispida, Chara curta, Chara contraria* and *Chara rudis* were also found in this division. Division 4 was also associated with a diverse range of *Potamogeton* spp, including*, Potamogeton pectinatus, Potamogeton rutilis, Potamogeton filiformis*, *Potamogeton pusillis* and *Potamogeton x nitens.* In subgroup H (Fig. 4.15) *N. flexilis* was associated with *Elodea nutallii, Equisitum fluviatile, Callitriche hermaphrodicia, Myriophyllum alterniflorum, Potamogeton perfoliatus* and *Potamogeton gramineus,* but most of these species were present in very low abundances at the sample points that contained *N. flexilis.*

Table 4.5 Macrophyte species associated with each TWINSPAN-derived division and subgroup, with indicator species underlined. Species are listed in order of abundance, greatest first. Only species with percentage cover >5% are shown (a full list can be found in Appendix 6)

Division	1				
Subgroup	A	B	G	**F**	**J**
Species	*Litorella uniflora* *Elodea canadensis* *Elatine hexandra* *Nitella flexilis* agg. *Eleocharis acicularis* *Myrophyllum alterniflorum* *Potamogeton berchtoldii* *Chara virgata* *Isoetes lacustris*	*Litorella uniflora* *Elodea canadensis* Freshwater sponge *Lobelia dortmana* *Juncus bulbosis* *Myriophyllum alterniflorum* *Isoetes lacustris*	*Elodea canadensis* *Litorella uniflora* *Nuphar lutea* *Potamogeton obtusifolius* *Chara virgata* *Nitella flexilis* agg. *Myriophyllum alterniflorum* *Persecaria amphibia* *Eleocharis acicularis* *Subularia aquatica* *Potamogeton berchtoldii* *Isoetes lacustris* *Potamogeton gramineus*	*Potamogeton berchtoldii* ***Najas flexilis*** *Sparganium angustifolium* *Potamogeton perfoliatus* *Chara* spp. *Elodea canadensis* *Juncus bulbosis* *Phragmites australis* *Schoenoplectus lacustris* *Isoetes echinospera* *Potamogeton gramineus*	*Potamogeton berchtoldii* ***Najas flexilis*** *Potamogeton perfoliatus* *Nitella flexilis* agg. *Nitella translucens* *Myriophyllum alterniflorum*

Division	2			4	
Subgroup	**C**	**D**	**E**	**H**	I
Sites	*Juncus bulbosis* *Litorella uniflora* *Potamogeton gramineus* *Potamogeton natans* *Eleogiton fluitans* *Lobelia dortmana* ***Najas flexilis*** *Phragmites australis* *Sparganium angustifolium* *Carex rostrata* *Chara virgata* *Apium inundatem* *Menyanthes trifoliata* *Potamogeton perfoliatus* *Utricularia vulgaris* agg. *Nitella transulcens* *Utricularia styggia* *Myriophyllum alterniflorum*	*Litorella uniflora* *Juncus bulbosis* *Lobelia dortmana* *Myriophyllum alterniflorum* ***Najas flexilis*** *Potamogeton gramineus* *Potamogeton perfoliatus* *Isoetes lacustris* *Utricularia vulgaris* agg. *Elatine hexandra* *Callitriche bruttia* *Potamogeton natans* *Nitella translucens* *Chara virgata* *Subularia aquatica* *Sparganium angustifolium* *Callitriche hermaphroditica*	*Juncus bulbosis* *Litorella uniflora* *Potamogeton gramineus* *Lobelia dortmana* ***Najas flexilis*** *Potamogeton perfoliatus* *Myriophyllum alterniflorum* *Sparganium angustifolium* *Chara virgata* *Isoetes echinospera* *Eleocharis palustris* *Potamogeton natans* *Equisitum fluviatile* *Phragmites australis*	*Elodea nuttallii* *Potamogeton gramineus* *Myriophyllum alterniflorum* *Potamogeton perfoliatus* *Chara aspera* *Litorella uniflora* *Potamogeton pusillis* *Chara virgata* *Potamogeton rutilis* *Eleocharis palustris* ***Najas flexilis*** *Potamogeton filiformis* *Potamogeton x nitens* *Potamogeton pectinatus*	*Chara aspera* *Myriophyllum alterniflorum* *Potamogeton gramineus* *Litorella uniflora* *Myriophyllum spicatum* *Eleocharis palustris* *Potamogeton pectinatus* *Potamogeton filiformis* *Equisitum fluviatile* *Potamogeton natans* *Potamogeton perfoliatus*

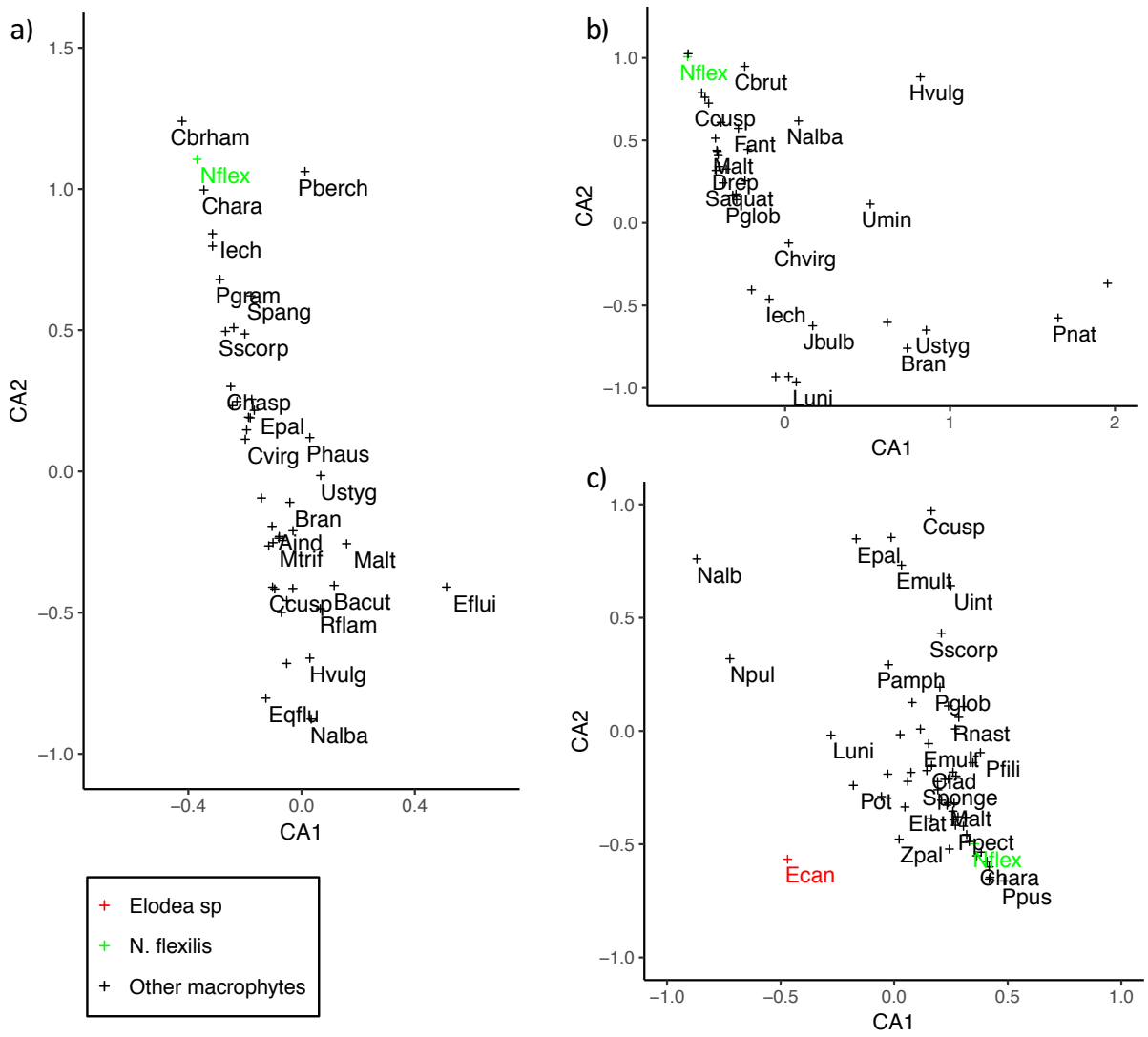

Figure 4.13 Correspondence Analysis plots for division 2 sites containing N. flexilis*: a) Subgroup c (plotted axes explain 12% of variance); b) subgroup d (plotted axes explain 14% of variance); c) subgroup e (plotted axes explain 8% of variance). Not all species shown (full species lists can be found in Appendix 6).*

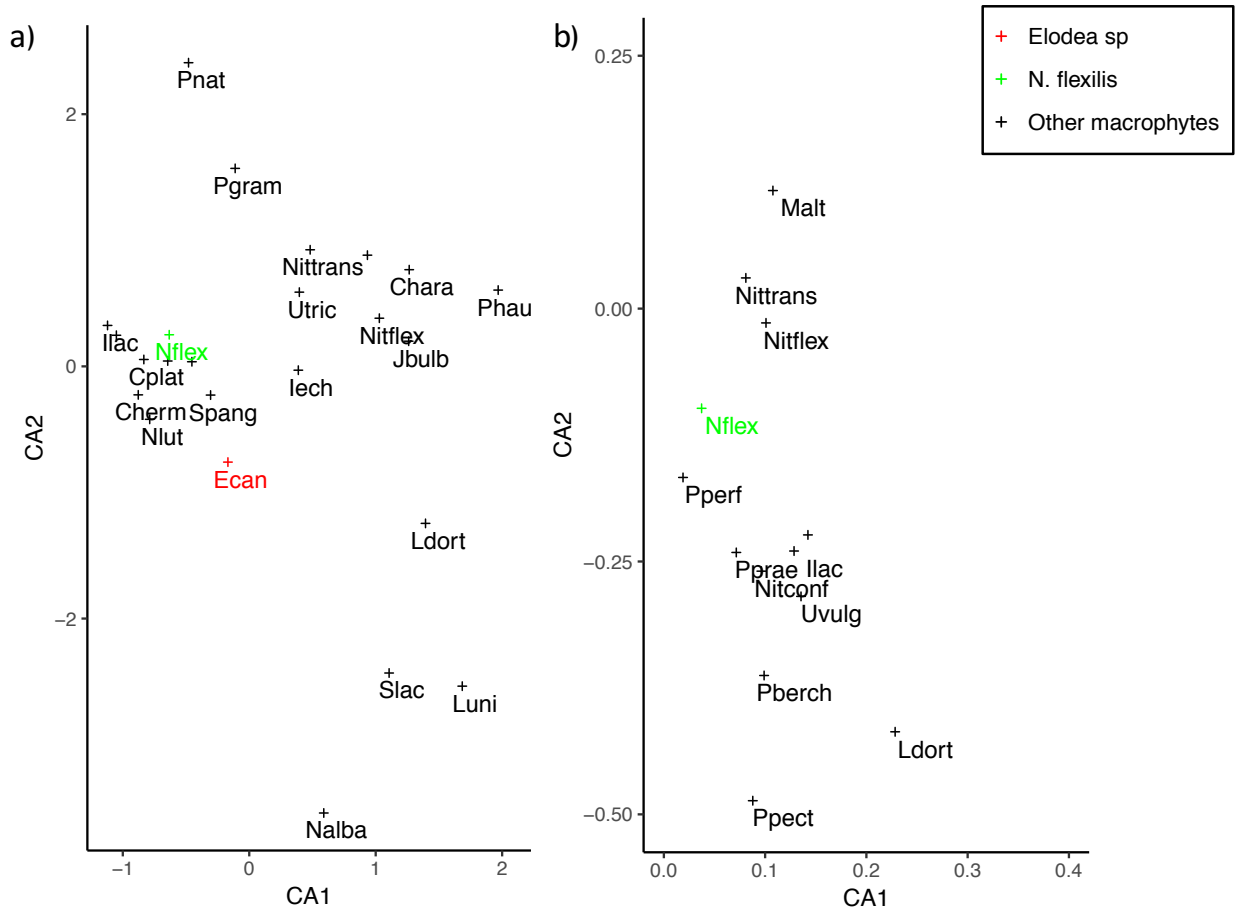

Figure 4.14 Correspondence Analysis plots for division 3 sites containing N. flexilis*: a) Subgroup f (plotted axes explain 21% of variance); b) subgroup j (plotted axes explain 22% of variance). Not all species shown (full species list and plots can be found in Appendix 6)*

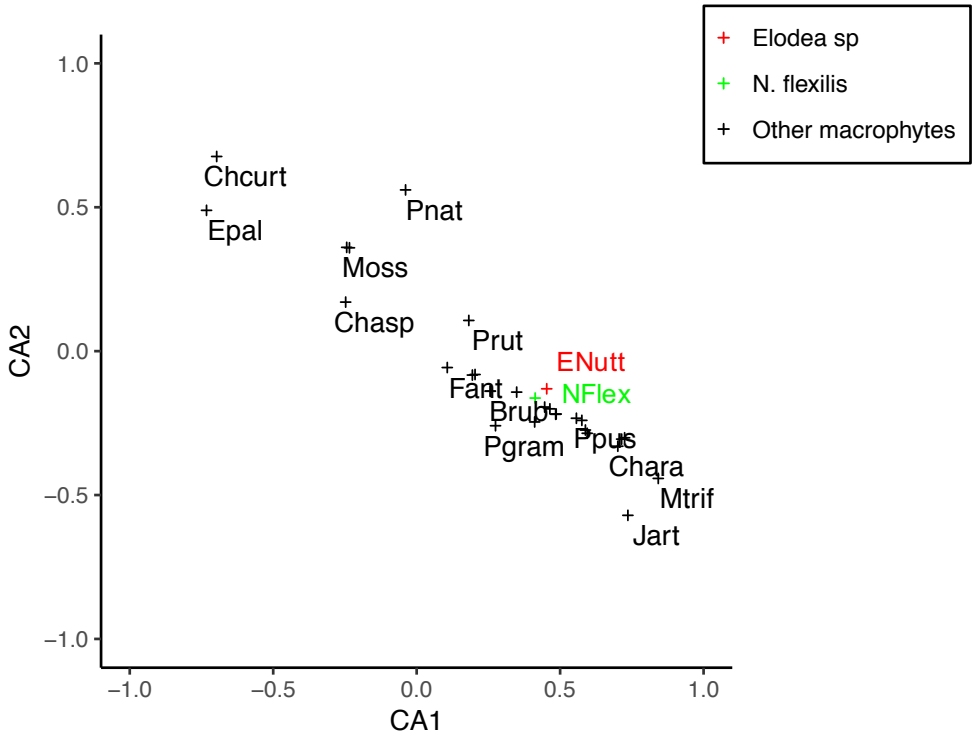

Figure 4.15 Correspondence Analysis plots for division 4 sites containing N. flexilis (i.e. subgroup h). Plotted axes explain 15% of variance. Not all species shown (full species list and plots can be found in Appendix 6)

It was not possible to conduct meaningful statistical tests for significant differences in water chemistry between the different TWINSPAN groups because of the small number of water chemistry data points for each group. Missing values in the water chemistry record also precluded the use of constrained ordination analyses. However, it was possible to draw out some differences using boxplots (Fig. 4.16). Alkalinity was below 30mg $CaCO_3$/l in most subgroups, with subgroup E having the lowest mean alkalinity (10.48mg/l). Division 4 subgroups (H and I) had relatively high alkalinities, with means of 51.65mg/l and 102.32mg/l respectively. All sites in subgroup F had a high LEC (the group mean was 3.08), whilst those in subgroups G and H had low LECs, with means of 1.20 and 1.32 respectively. Subgroups A, D, G and I had nitrate concentrations below 0.015 mg/l, whilst division 3 subgroups (F and J), despite still having low nitrate concentrations (below 0.15 mg/l), had the highest of all groups. Mean pH was above 8 in subgroups A, G, H and I, and below 8 in all other subgroups. Salinity was below 0.1ppm in all subgroups, but, of the subgroups for which records were available, was highest in

subgroup F and lowest in subgroup H. There was little variation in TDS between subgroups, except for subgroup H, where TDS was much higher than all other groups at 213ppm. Total P was low (below 0.03mg/l) at all sites, but, within this range, was highest (means above 0.02mg/l) in subgroups A, F, H and I, and lowest (means below 0.01mg/l) in subgroups D and E.

Division 1 sites were characterised by high wind exposure. In Mann-Whitney tests, the only subgroups for which there was a significant difference in mean exposure between sample points where *N. flexilis* was present and absent were subgroups D and E ($W = 14854$, $P = 2.91 \times 10^{-3}$ and $W = 39550$, $P = 9.08 \times 10^{-9}$ respectively, Fig. 4.17). In subgroup D, *N. flexilis* was found in areas of high exposure, whilst in subgroup E it was found in areas of low exposure.

Significant differences were found between the mean depth of sample points where *N. flexilis* was present and sample points where *N. flexilis* was absent in all subgroups (Fig. 4.18). In divisions 2 and 4 (subgroups C, D, E, H and I), *N. flexilis* was found in the deepest parts of the photic zone. In division 3, *N. flexilis* was found towards the shallower end of the photic zone. *N. flexilis* was growing significantly deeper in subgroup J than in subgroups C and E, and mean *N. flexilis* depth in subgroup H was significantly deeper than in subgroup E. According to a Pearson's chi-squared test for association, there were no significant differences in the types of substrate on which *N. flexilis* was found between subgroups.

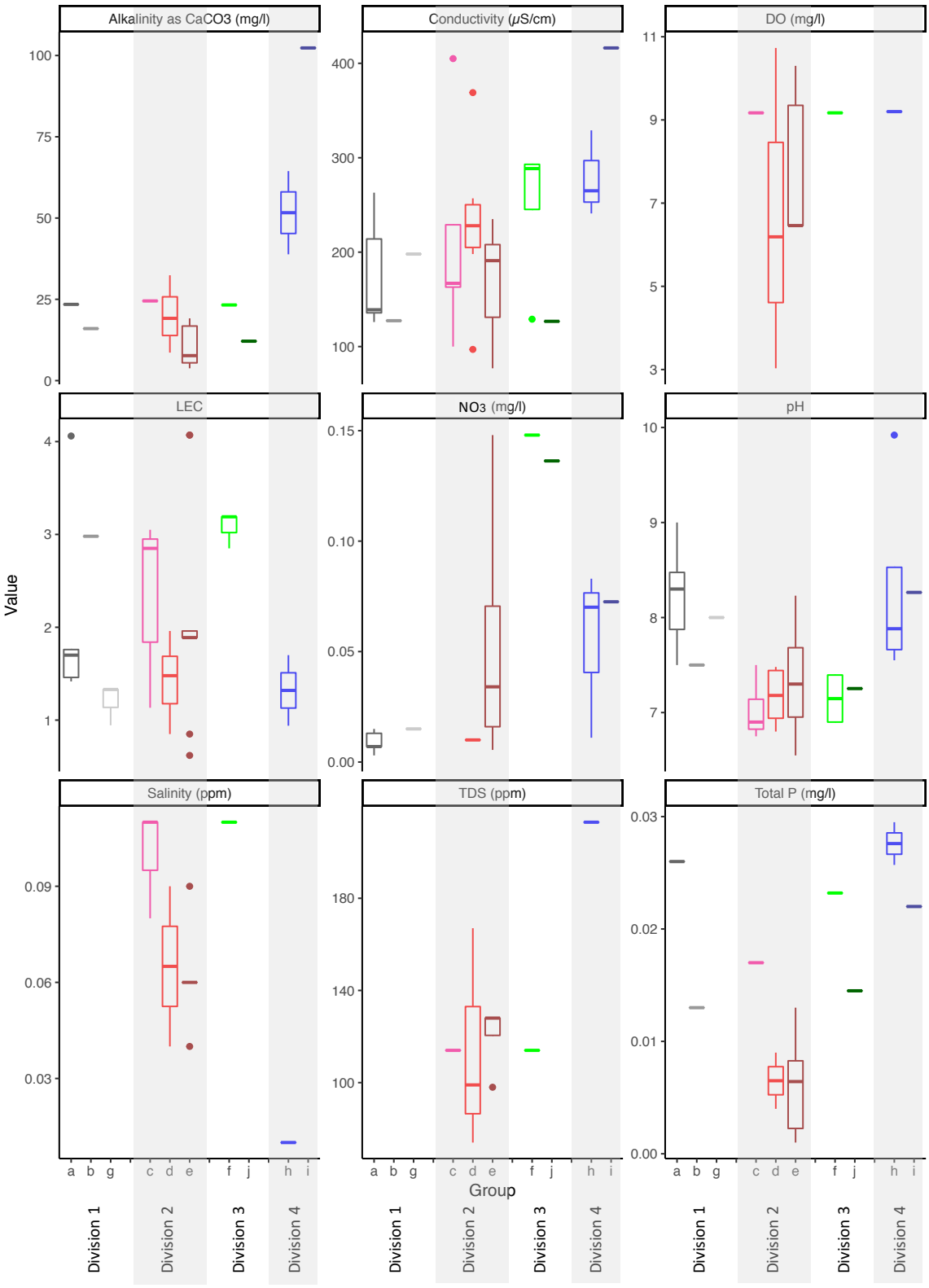

Figure 4.16 Boxplots comparing water chemistry variables of TWINSPAN groups.

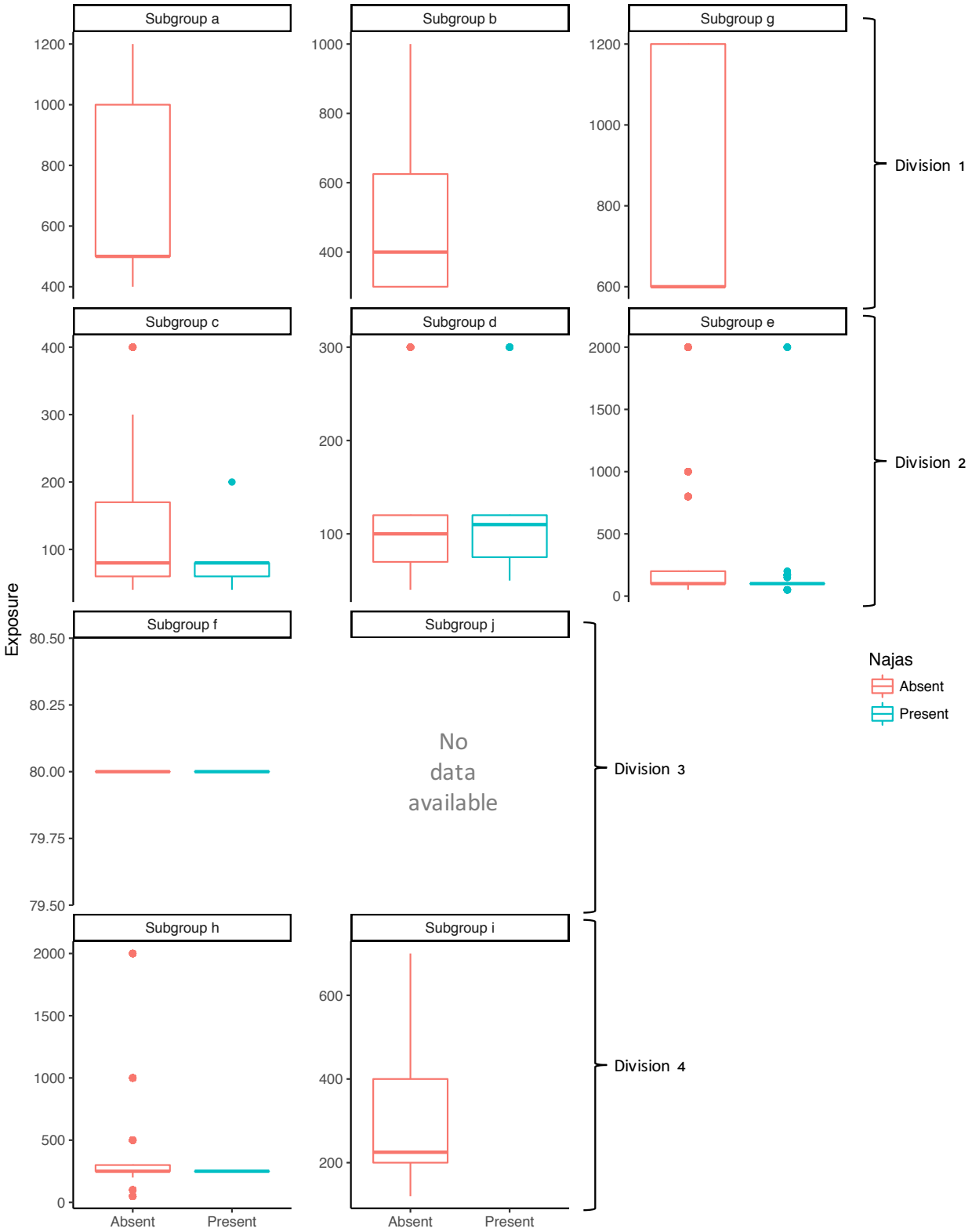

Figure 4.17 Boxplots comparing wind exposure of sample points where N. flexilis *was present (blue) and absent (red) across the different TWINSPAN groups.*

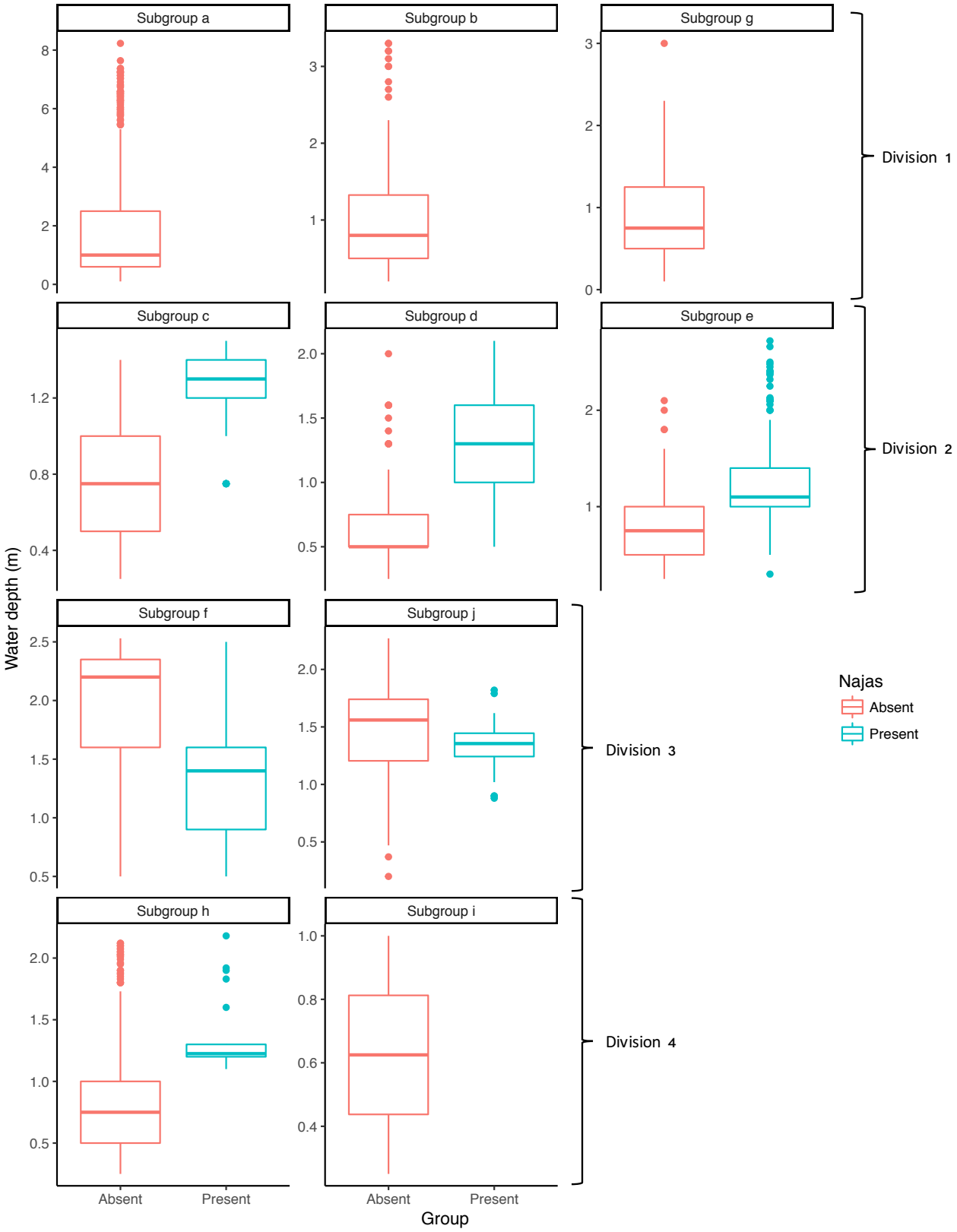

Figure 4.18 Boxplots comparing depth of sample points where N. flexilis was present (blue) and absent (red) across the different TWINSPAN groups.

4.2.4 Do *Elodea* spp. directly compete with *N. flexilis* within the same habitat?

Of the 26 Scottish sites considered here, 12 contained either *Elodea canadensis* or *Elodea nuttallii* during the most recent survey. Since 2000, *Elodea* spp. have been newly recorded at two sites, both in the Western Isles; Loch an Eilean and Loch Druidibeg. Of the ten sites at which *N. flexilis* was found formerly but is not currently present, seven now contain *Elodea* spp. *Elodea* spp. are present at sites in Mainland Scotland, the Inner Isles and the Western Isles. A Kendall's Tau test showed a significant negative correlation between the percentage of sample points per survey occupied by *N. flexilis* cover and *Elodea* spp. percentage cover ($\tau = -0.3124$, $P = 0.01$). However, in principal components analyses of plant associations on both the lake-wide and micro-habitat scales (Figs. 4.4 and 4.8 respectively), *Elodea* spp. were not closely associated with *N. flexilis.*

Elodea is present in all divisions identified in the TWINSPAN analysis (Fig. 4.19). Divisions 1, 2 and 3 are associated with *Elodea canadensis,* whilst division 4 is associated with *Elodea nuttallii.* Division 2 has the lowest abundance of *Elodea* spp; only 3% of sample points in subgroup E contained *Elodea canadensis.* All other divisions contained *Elodea* spp. in abundances of up to 40% per subgroup. In subgroups where both species were present, *N. flexilis* did not appear to be closely associated with *Elodea* spp. in correspondence analyses, aside from in subgroup F (division 3).

Sites at which *Elodea* spp. were found had significantly higher pH and total phosphorus concentrations than those at which *N. flexilis* was present ($W = 137$, $P = 0.001$ and $W = 17.5$, $P = 0.05$ respectively). Nitrate concentrations were significantly lower at sites containing *Elodea* spp. than at sites containing *N. flexilis* ($W = 113.5$, $P = 0.01$). No significant differences were found between sites containing *Elodea* spp., and those containing *N. flexilis* for other water chemistry variables (Fig. 4.20).

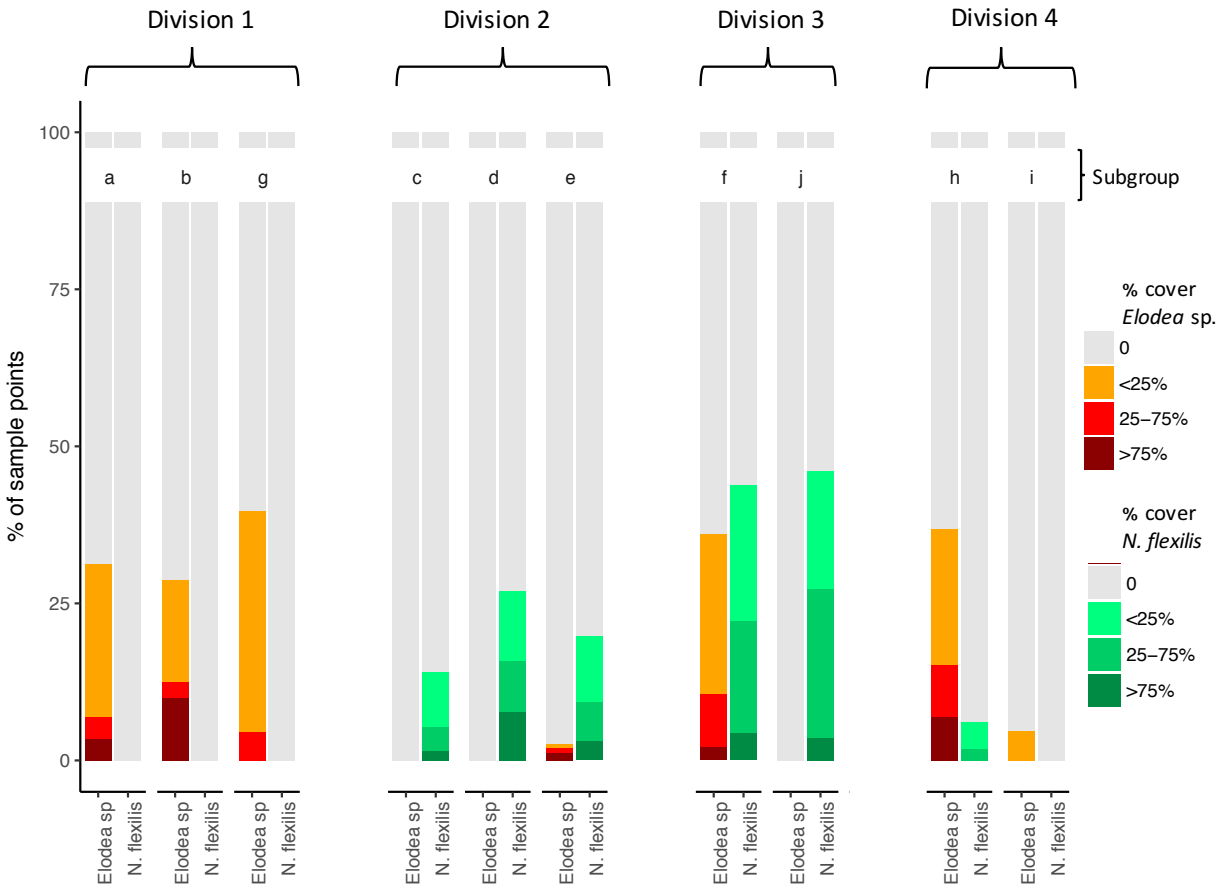

Figure 4.19 Percentage of sample points in each TWINSPAN group in which N. flexilis *and* Elodea *spp. percentage covers were <25%, 25 - 75%, and >75%.*

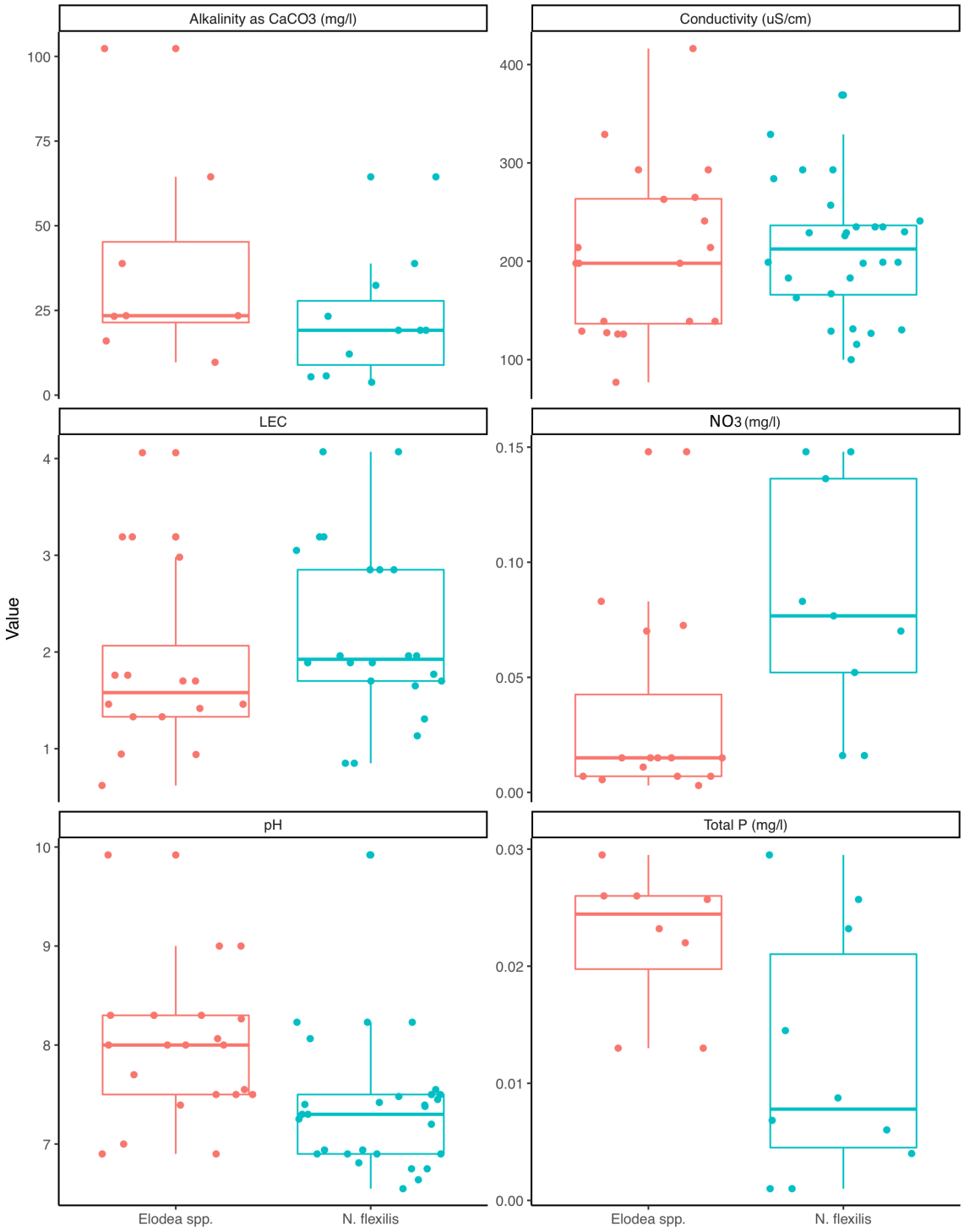

Figure 4.20 Boxplots comparing water chemistry variables of lakes containing N. flexilis *and lakes containing* Elodea *spp.*

Samples containing *Elodea* spp. and those containing *N. flexilis* were found within the same water depth range, with no significant difference between means of the two groups in a Mann-Whitney test (Fig. 4.21a). *N. flexilis* was found in areas of significantly lower exposure than *Elodea* spp. ($W = 151960$, $P < 2.2 \times 10^{-16}$), however Fig. 4.21b shows some overlap between the two groups. There was no significant difference in the distribution of substrate types between *Elodea* spp. sample points and sample points where *N. flexilis* was present ($X^2 = 10.439$, $P = 0.11$), with both species found predominantly on silt and sand substrates (Fig. 4.22).

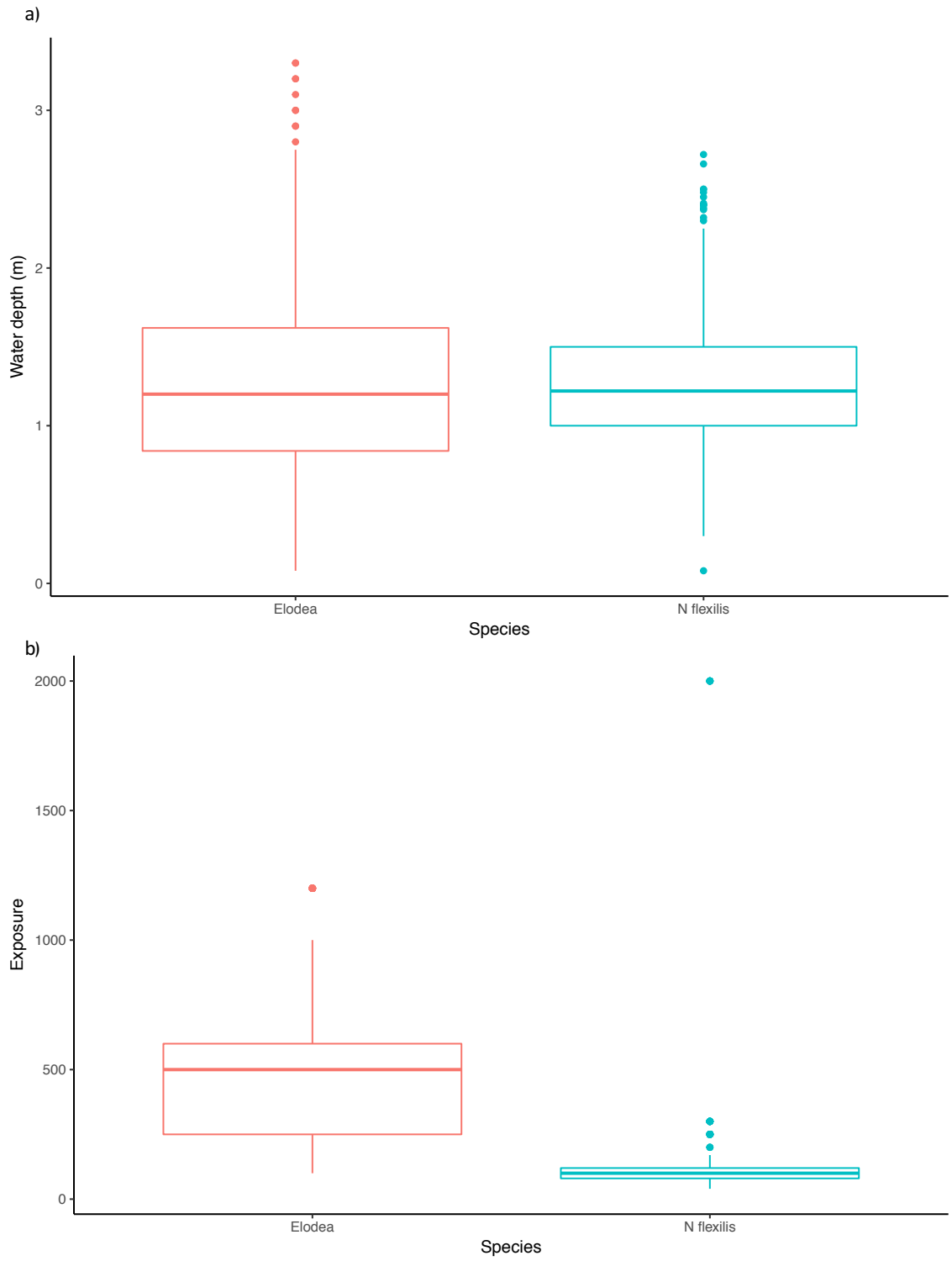

Figure 4.21 Boxplots comparing a) water depth and b) exposure of sample points containing N. flexilis *and those containing* Elodea *spp.*

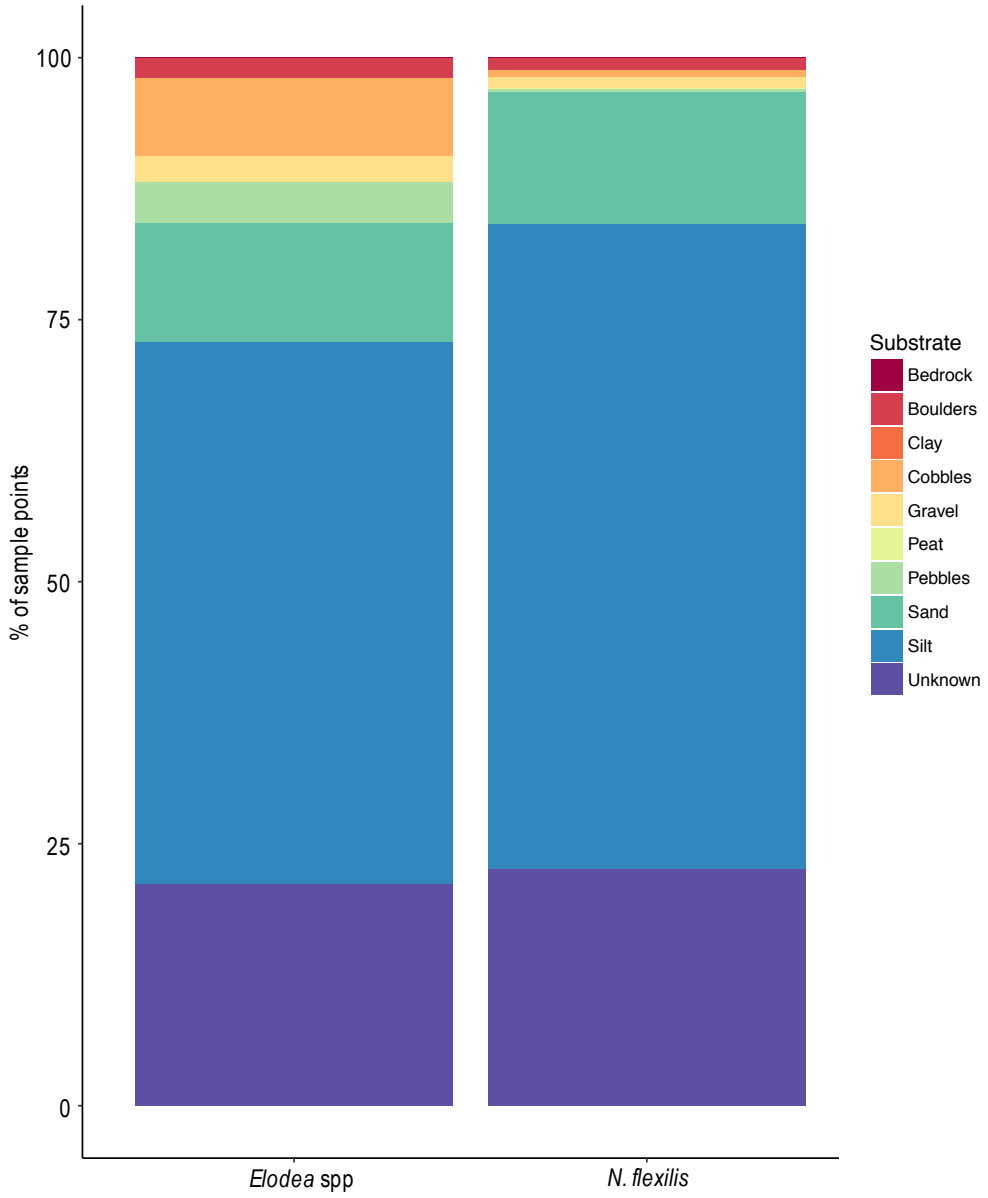

Figure 4.22 Substrate types at sample point containing N. flexilis and sample points containing Elodea spp.

4.3 Discussion

4.3.1 To what extent are *N. flexilis* populations in the British Isles in decline?

N. flexilis was first recorded in Ireland in 1851. Compilation of all records of *N. flexilis* in

the British Isles since this time reveals that a total of 125 sites have, at some time, contained the plant. Wingfield *et al.* (2004) reported 79 known *N. flexilis* sites for the period 1998 – 2004. Since this study, *N. flexilis* has been found at a further two sites in Scotland and at ten sites in Ireland. In recent years, past records for a further 25 Irish sites have been uncovered by Roden (2004), and one past record for an additional Scottish site (Loch na Paice Dubh) was discovered during the course of this research. Although not included in the results presented here, a paleolimnological record of *N. flexilis* seeds was also found from Loch Eye, Ross, in the Scottish Highlands, with seeds present throughout an undated core of 75cm length (Birks and Sayer, unpublished data). These new records are more likely to be the result of an increased intensity of survey since the introduction of the Water Framework Directive and the European Habitats Directive in 2000. However, 27 sites at which *N. flexilis* has formerly been identified are not subject to regular survey, and the current status of the plant at these sites remains unknown.

N. flexilis has not been found at 32 of the 125 sites where it has formerly been recorded in surveys during the last ten years; 16 of these sites are in Ireland, 15 in Scotland and two in England. The plant was last recorded in what is assumed to have been its last English locale (Esthwaite Water, Cumbria) in 1982. In Scotland, most losses have occurred on the mainland, where *N. flexilis* disappeared from 11 of its 17 locales during the 2000s. *N. flexilis* was also absent from four sites in the Western Isles during recent surveys; it was last seen in Loch Bun an Ligidh, West Loch Ollay, and at an unnamed loch on South Uist in the 2000s, and in Loch Grogary in 2010. It was also only found in very small numbers in Loch Scaraidh (North Uist) in 2016 following an intensive snorkel survey, having been recorded as absent at the site in 2010. Although evidence from the SCM surveys suggests that *N. flexilis* abundance at a site can fluctuate from year to year, all the sites at which *N. flexilis* was absent during the most recent survey have long records of *N. flexilis* presence, dating from the 1870s on the mainland and the 1940s in Western Isles sites. The Scottish Islands have previously been indicated as relatively un-impacted by human activity (McCloskey and Spalding, 1989), but it is clear that *N. flexilis* populations are now in decline even in these remote regions.

4.3.2 Under what conditions does *N. flexilis* currently grow in the UK?

Water chemistry variables have been presented here as annual means. For most sites included in this analysis, these means were calculated from the results of between one and eight water samples collected from the centre of the lake in each year. Although it is recognised that this sampling frequency is not adequate for capturing the full range of environmental conditions present in any single growing season, the differences in water chemistry that were identified between the sites were consistent with differences in the vegetative communities present. For example, lakes with the highest measured mean annual alkalinities were characterized by *Chara* spp and *Potamogeton* spp, which are considered typical of base-rich lakes (Duigan *et al.*, 2006). On the basis that there was broad agreement between the water chemistry results and the flora recorded, water chemistry results were considered adequate to draw conclusions about the current UK habitat of *N. flexilis.*

N. flexilis was associated with sites with circumneutral pH, whilst sites where *N. flexilis* was absent had higher pH. There was overlap in the ranges of the two groups, with *N. flexilis* being found in one site (Loch Scaraidh in 2016) with a pH of 9. Nevertheless, the highest percentage covers of *N. flexilis* were found at lakes with a circumneutral pH. None of the sites included in the study had a pH lower than 6.5, so the lower end of the tolerance range of *N. flexilis,* which, according to laboratory studies is believed to be between 5 and 6, was not observed (Titus and Hoover, 1993). No correlation was found between *N. flexilis* presence/abundance and alkalinity, despite a known link between alkalinity and *N. flexilis* carbon fixation (Wetzel and McGregor, 1968). This may be because lakes with measured alkalinities of 3 – 102 mg $CaCO_3$/l were included in the study, which may span both the lower and upper tolerance ranges of *N. flexilis.* If this is the case, differences in lakes where *N. flexilis* was present and those where it was absent would not be easily detected in tests for differences in means of groups defined by *N. flexilis* presence and absence. Wingfield *et al.* (2004) found that *N. flexilis* presence was correlated with concentrations of magnesium, calcium, alkalinity and pH, with *N. flexilis*

absent in lakes at both the acidic and alkaline extremes. This conclusion was based upon splitting sites into three groups: those at which *N. flexilis* was present, those at which *N. flexilis* was absent and appeared oligotrophic, and those at which *N. flexilis* was absent and appeared mesotrophic. This approach was not repeated in this study because it was difficult to tell whether it produced a "true" finding or the result was guaranteed by the method.

Measurements of nutrient concentrations were mostly based on single samples taken during the summer, when nutrient uptake from plants is high, so the ranges for both total phosphorus and nitrate concentrations were close to the lower detection limits for all sites. Chlorophyll-a data was not available for any site. Within the small range of measured nutrient concentrations, *N. flexilis* was present in sites with relatively low total phosphorus concentrations and relatively high nitrate concentrations. *N. flexilis* abundance was also positively correlated with nitrate concentrations. Wingfield (2002) also found a significant difference in phosphorus concentrations between the "*N. flexilis* present" group and the "absent eutrophic" group. Since phosphorus often limits primary production in mesotrophic lakes, higher concentrations of phosphorus increase photosynthesis, leading to increased consumption of carbon dioxide within a lake (Schindler and Fee, 1973). *N. flexilis* is known to take up phosphorus from sediments, so can photosynthesise in situations where phosphorus concentrations in the water are lower (Moeller *et al.*, 1988). Furthermore, *N. flexilis* is an obligate carbon dioxide user and, unlike many plants found in hard water environments, it cannot use bicarbonate for photosynthesis (Hough and Wetzel, 1978; Hough and Fornwall, 1988). This gives it a competitive disadvantage in lakes with elevated concentrations of phosphorus in the water column. The correlation between nitrate and *N. flexilis* presence/abundance found here suggests that nitrogen rather than phosphorus may limit *N. flexilis* growth, but this is less widely reported in the literature. In a laboratory-based study of *N. flexilis* phosphorus uptake from lake sediments, Moeller *et al.* (1988) suggested that the high concentrations of phosphorus found in *N. flexilis* shoots implied that phosphorus was not limiting growth, but a limiting factor was not determined. In Shoe Lake, Minnesota, a drought event that reduced nutrient inflow into a nitrogen-limited system was associated with

increased growth of all rooted macrophytes except for *N. flexilis* and *Myriophyllum verticallatum*, whose abundances declined when nitrogen concentrations reduced (Tracy *et al.*, 2003). This was attributed to increased water clarity during a drought year, which favoured bicarbonate-using plants because the energy required for active transport of bicarbonate requires higher photosynthetic rates. Such a hypothesis is supported by this study, where *N. flexilis* was found at higher abundances at sites where light penetration was low, and was also found at sample points with greater water depths when all sample points were considered collectively.

Across all sites, *N. flexilis* was found growing at a range of water depths, from a minimum of 0.3m to a maximum of 2.72m. However, the water depth of *N. flexilis* with relation to other macrophytes varied across sites, with *N. flexilis* growing in shallow waters relative to other plants in some lakes and in deeper waters in others. This is consistent with research on the species in Ontario, Canada, where *N. flexilis* has been observed growing in water depths from 0.2 – 14.0 m (Pip and Simmons, 1986). Several studies have shown that the distribution of aquatic macrophytes within lakes is determined by the interaction between light penetration and the availability of organic carbon (e.g. Madsen and Sand-Jensen, 1994; Vestergaard and Sand-Jensen, 2000; Van den Berg *et al.*, 2002). Most aquatic plants thrive where light availability is high, and, in alkaline environments, most macrophytes overcome the low availability of CO_2 by using bicarbonate for photosynthesis (Maberly and Spence, 1983). *N. flexilis* is not able to use bicarbonate, but compensates for this by being tolerant of low light conditions, allowing it to grow in deeper areas of the lake where photosynthetic rates are lower and CO_2 is therefore more likely to be available. Thus, the depth at which *N. flexilis* is found at any individual site likely reflects the balance between alkalinity, light penetration, and competition from other plants (which, in turn, is linked to nutrient availability). This balance is likely different at different sites, explaining the wide range of water depths at which *N. flexilis* was found.

Exposure, which was used as a proxy for disturbance in this study, was found to correlate with the depth of *N. flexilis* occurrence. It is worth noting that exposure is not a measure

of all types of disturbance, since it only includes the action of wind-driven waves and excludes other causes of disturbance, such as animal activity or sediment slumping. Regardless, *N. flexilis* grew in deeper waters where exposure, and therefore disturbance from waves, was low. This agrees with the findings of Wingfield (2002), who found exposure to be significantly lower in sites where *N. flexilis* was growing at water depths <1m than those where it was growing in depths >2m. Since *N. flexilis* was found growing on all substrate types and in fluid and stable sediments alike, it is unlikely that differences in *N. flexilis* water depth are linked to the effect that exposure has on sediment texture. Rather, *N. flexilis* seems to prefer to live in areas that are subject to less disturbance. This opposes observations from Thrush Lake, Minnesota, where *N. flexilis* grows in areas disturbed by fishing tackle (Hagley *et al.*, 1996). However, these observations were made following the liming of the formerly oligotrophic lake, when *N. flexilis* would likely have been competing with bicarbonate-using plants. In this situation, it is possible that *N. flexilis* may have been unable to compete in less disturbed areas of the lake due to competition from other plants.

The interactions between water chemistry and the micro-habitat occupied by *N. flexilis* can be explored further by examining the characteristics of the groups identified by the TWINSPAN model (summarised in Table 4.6). Division 1, which contained only former *N. flexilis* sites and was typified by transect diagrams containing dense stands of *Elodea canadensis*, had the highest concentrations of phosphorus and the lowest concentrations of nitrogen. It also had a plant community typical of mesotrophic conditions. Many of these species, particularly *E. canadensis* and *Nitella flexilis* agg., preferentially use bicarbonate for photosynthesis in conditions of mid-high alkalinity (Allen and Spence, 1981). Although the measured alkalinity at these sites was relatively low, many measurements were taken in the middle of the day in summer, when photosynthetic rates were high. During the day, photosynthesising plants consume CO_2 or bicarbonate and release oxygen, which binds with hydrogen ions in the water, leading to an increase in pH. pH at division 1 sites ranged from 7.5 – 9, which may reflect the fact that bicarbonate was being consumed during photosynthesis by plants such as *E. canadensis* and *Nitella flexilis* agg. At these sites, where higher phosphorus concentrations were present, it is

likely that *N. flexilis* was unable to compete with species that are adept at survival in conditions where increased primary productivity causes CO_2 to be rapidly consumed. An alternative hypothesis for the absence of *N. flexilis* at these sites is that its growth was limited by low nitrate concentrations, however it is unclear whether low measured nitrate was due to the collection of measurements at a time when nitrate consumption by plants was likely to be high. Long-term regular water quality monitoring from the Lunan Burn – a small river which connects all the division 1 sites – suggests that concentrations of both NO_2 and NO_3 in the river downstream of Loch of Lowes were higher in the late 1990s and early 2000s than today (these monitoring records are described in more detail in chapter 6). It was during this period that *N. flexilis* was last recorded at these sites. To determine whether the ratio of carbon dioxide to bicarbonate or the low availability of nitrogen is responsible for the lack of *N. flexilis* at division 1 sites, these water chemistry variables need to be monitored over a longer time period.

Division 2 contained a mix of sites at which *N. flexilis* was currently present and those at which it was formerly present. *N. flexilis* percentage cover at these sites ranged from 0 – 40%, although most sites were within the 10 – 25% range. These sites had low phosphorus concentrations but relatively high NO_2 concentrations. They had alkalinities <35mg CaCO3/l, and circumneutral pH. Their floras were incredibly diverse, and reflective of mesotrophic conditions. They contained species tolerant of mildly acidic conditions, such as *Juncus bulbosis* and *Sparganium angustofolium* (Hill and Ellenberg, 1999). Many of these sites were located on the Machair sands of the Western Isles, where alkalinity derived from the underlying, base-rich geology is offset by acid runoff from their highland catchments (Wingfield *et al.*, 2006). At these sites, *N. flexilis* was found growing at a range of water depths, in areas of low and high exposure, and alongside many other plant species; transect diagrams showed *N. flexilis* growing either in dense, mono-specific stands, or scattered more sparsely throughout the photic zone This is likely because both alkalinity and phosphorus concentrations are low enough not to create a competitive advantage to bicarbonate users, but nutrients – particularly nitrate concentrations – are high enough to fuel plant growth. pH is also high enough not to inhibit the reproductive capabilities of *N. flexilis.* All sites within this division where *N.*

flexilis was absent had pH <7.5.

Division 3 sites all contained high abundances of *N. flexilis*, ranging from 30 to 46% cover. Chemically, they were very similar to division 2 sites, but their flora was reflective of slightly more alkaline conditions (Hill and Ellenberg, 1999). *Juncus bulbosus,* for example, was less abundant at these sites than at division 2, whilst *Potamogeton perfoliatus, Chara* spp. and *Nitella* spp. were more abundant. Here, *N. flexilis* was found at a wider range of water depths, but, in contrast to division 2, was commonly associated with a specific area within the depth zonation, often growing alongside *Potamogeton perfoliatus*. Although *P. perfoliatus* is an efficient bicarbonate user (Vestergaard and Sand-Jensen, 2000), levels of CO_2 in the water are high enough for *N. flexilis* to remain competitive with this species at these sites. This may be linked to low phosphorus concentrations limiting the primary productivity of bicarbonate users. Division 3 sites had relatively low light penetration, with a maximum LEC of 3.2 compared to 4.1 in division 2. *N. flexilis* was found living in water depths up to 2.5m, where light availability was low. This is likely because *N. flexilis* tolerates low light conditions well (Wingfield, 2002), giving it a competitive advantage in the deeper areas of a lake.

Division 4 contained a mix of sites where *N. flexilis* was absent and sites where *N. flexilis* was present at very low abundances (<6% cover). All lakes were base-rich, with alkalinities up to 102mg $CaCO_3$/l and with pH up to 10. The lakes were also relatively nutrient rich in terms of both phosphorus and nitrates, although phosphorus concentrations were not as high as in division 1 sites. Plant composition was reflective of the high alkalinity, with an extremely diverse Charophyte flora present; *Chara* spp. are particularly tolerant of alkaline waters (Maberly and Spence, 1983). At these sites, *N. flexilis* only grew in deep water (>1.1m), usually below the maximum colonisation depth of all other species. This is because CO_2 levels are higher below the photic zone in lakes with high alkalinities, where it is not being consumed by photosynthesis. Since *N. flexilis* can tolerate low light conditions, it can survive at greater depths than other plants, allowing it to exploit the higher CO_2 levels found in these areas. Plants associated with *N. flexilis* in this zone were similarly tolerant of low light levels; *Elodea nuttalli,* for example, is known to adapt to low light levels by producing taller plants with longer

leaves (Simpson, 1988).

Table 4.6 Summary characteristics of sites belonging to each TWINSPAN division.

Division	**1**	**2**
N. flexilis abundance	Absent	Medium (10-25% cover)
Water chemistry	Alkalinity <25 mg CaCO3/l Conductivity 120 – 260 uS/cm LEC 0.9 – 4 NO2 < 0.015mg/l pH 7.5 – 9 Total P > 0.01mg/l	Alkalinity <35 mg CaCO3/l Conductivity 70 – 410 uS/cm LEC 0.6 – 4.1 NO2 0.01 – 0.15mg/l pH 6.5 – 8.25 Total P < 0.01mg/l
Characteristic species	*Elodea canadensis, Nitella flexilis* agg., *Isoetes* spp.	*Juncus bulbosus, Potamogeton gramineus, Potamogeton natans, Sparganium angustifolium, Utricularia* spp.
N. flexilis water depth	N/A	0.3m – 2.72m
N. flexilis exposure	N/A	40 – 2000
Species associated with *N. flexilis*	N/A	*Callitriche* spp.
Division	**3**	**4**
N. flexilis abundance	High (30 – 46% cover)	Low (6% cover) or absent
Water chemistry	Alkalinity <25 mg CaCO3/l Conductivity 125 – 300 uS/cm LEC 2.8 – 3.2 NO2 > 0.1mg/l pH 6.9 – 7.4 Total P < 0.02mg/l	Alkalinity 35–102 mg CaCO3/l Conductivity 240 – 420 uS/cm LEC 0.9 – 1.7 NO2 0.01 – 0.09mg/l pH 7.5 – 10 Total P 0.02 – 0.03mg/l
Characteristic species	*Najas flexilis, Potamogeton perfoliatus, Potamogeton berchtoldii*	*Chara* spp.
N. flexilis water depth	0.5m – 2.5m	1.1 – 2.2m
N. flexilis exposure	80	250
Species associated with *N. flexilis*	*Potamogeton perfoliatus*	*Elodea nutallii, Equisitum fluviatile, Callitriche hermaphroditica, Myriophyllum alterniflorum, Potamogeton perfoliatus, Potamogeton gramineus.* Low abundance.

4.3.3 How directly do *Elodea* spp. compete with *N. flexilis* within the same habitat?

Although a negative correlation was found between *N. flexilis* percentage cover and *Elodea* percentage cover, the two species did not appear to be associated with each other in principal components analyses, and there were many sites at which both species seemed to co-exist. This finding is identical to the observations of Wingfield (2002), who were unable to find any sites at which *Elodea* spp. were clearly outcompeting *N. flexilis,* despite informal reports of competition between the two species at Tangy Loch. In terms of micro-habitat, *Elodea* spp. occupy the same depth zone and silty substrates as *N. flexilis.* However, the range of substrate types and water depths tolerated by *Elodea* spp. was greater than *N. flexilis*, meaning that the two species are potentially able to coexist within different zones of the same lake. *Elodea* spp. often outcompete native plants for light and space by forming dense stands, and these dense stands were not observed within *N. flexilis* habitat at any of the lakes at which the two species were both present except for Loch Scaraidh. Here, dense stands of *Elodea nuttallii* and large unvegetated areas were occupying fine substrates in the water depths where *N. flexilis* was present. The reason for the absence of *N. flexilis* from these bare areas remains unexplained, but it seems unlikely that competition from dense stands of *Elodea* spp. fully explains the low abundance of *N. flexilis* at Loch Scaraidh. The introduction of *Elodea* spp. to Loch Scaraidh is relatively recent (it was first recorded in the Western Isles in the early 21st century), whilst *Elodea canadensis* has been present at some mainland sites since the 1950s (National Biodiversity Network, 2014). Dense stands of *Elodea* spp. are subject to decline and collapse following initial establishment, allowing the recovery of native macrophytes (Simberloff and Gibbons, 2004; Zehnsdorf *et al.*, 2015). Given the absence of long-term records of *Elodea* abundance at many *N. flexilis* sites, it is impossible to know whether dense stands may develop at sites at which the two species co-exist, or whether existing stands (e.g. at Loch Scaraidh and Tangy Loch) may collapse and allow new *N. flexilis* growth to occur.

Phosphorus concentrations were significantly higher in sites containing *Elodea* spp. than those containing *N. flexilis.* Indeed, former *N. flexilis* sites which now have relatively large abundances of *Elodea* spp. (Division 1 sites) have some of the highest measured total P concentrations. According to the literature, many of these sites (e.g. the Dunkeld-Blairgowrie lochs) have been subject to eutrophication (Finney, 1998; Bennion *et al.*, 2008; Bennion *et al.*, 2010). Transect diagrams from these sites showed dense stands of *Elodea canadensis* occupying *N. flexilis* habitat. Elevated phosphorus concentrations are known to promote biomass formation in *Elodea* spp. (Eugelink, 1998), and thus *Elodea* spp. invasion often co-occurs with eutrophication (Melzer, 1999). It is possible, therefore, that the correlation between *N. flexilis* percentage cover and *Elodea* spp percentage cover actually represents the individual responses of both species to phosphorus. It is not possible from the data presented here to definitively determine whether *Elodea* spp. have a direct negative impact on *N. flexilis,* or whether the interactions between the two species are environmentally dependent.

4.3.4 What are the likely causes of *N. flexilis* decline?

Similar to Wingfield (2002), this study found significant overlap in the conditions of current and former *N. flexilis* sites. The absence of *N. flexilis* from some sites can be attributed to a combination of high phosphorus concentrations, low pH, high alkalinity and competition from *Elodea* spp. All the sites within division 1 of the TWINSPAN analysis were former *N. flexilis* sites located on mainland Scotland; the last known record of *N. flexilis* at any division 1 site was from Loch of Lowes in 2007 (Benthic Solutions, 2007). All of these sites have been subject to anthropogenic eutrophication from catchment runoff, with additional phosphorus inputs at Loch of Butterstone from the Butterstone Trout Fishery and at Loch of Lowes from sewage discharge from a Scottish Wildlife Trust visitor centre (Bailey-Watts *et al.*, 1992; Ferrier *et al.*, 1997; Finney, 1998). It is likely that increases in primary productivity at these sites favoured bicarbonate-using plants, particularly *Elodea canadensis.* This is compounded by the fact that the efficiency of *Elodea canadensis* as a bicarbonate user increases as nutrient concentrations in the water increase (Madsen and Sand-Jensen, 1987). For division 2,

sites from which *N. flexilis* was absent had mildly acidic – circumneutral pH. Except for Lake of Menteith, these sites are located on either the Inner Hebrides or the Western Isles. Based upon "critical loads analysis", it is highly likely that acid deposition to lakes in these regions will exceed their acid buffering capacity (a function of catchment geology, soil composition and land use), meaning that they are at high risk of acidification (Kernan *et al.*, 2004). Since *N. flexilis* cannot reproduce at low pH, acidification could potentially cause a loss of the species at these sites (Titus and Hoover 1991; 1993). In division 3 sites, which have high alkalinities, *N. flexilis* can only survive in deep water where it is less likely to face competition from bicarbonate-users. However, *Elodea* spp. can thrive at the same water depths as *N. flexilis* by growing tall plants with long leaves, giving it the potential to shade out *N. flexilis* (Simpson, 1988). Furthermore, as a bicarbonate-user, *Elodea* spp. have a significant competitive advantage over *N. flexilis* at base-rich sites. *N. flexilis* populations in division 3 sites, therefore, may be at particularly high risk from *Elodea* spp. invasion.

For most sites at which *N. flexilis* was not present during the most recent survey, particularly those in division 1, SCM data from years in which *N. flexilis* was present were not available. The only exception to this is Loch Grogary, where *N. flexilis* was present in 2004 and 2010 but absent in 2016. The species composition of Loch Grogary in all three of these years placed it in division 4, meaning that no major floristic changes were identified between surveys. The evidence presented here suggests that the main threat to *N. flexilis* in division 4 groups is *Elodea* invasion, and this is reflected in the fact that *Elodea nuttallii* was abundant in all three surveys from Loch Grogary. Nevertheless, for most sites, it is not possible to identify chemical or floristic changes indicative of eutrophication, acidification, or *Elodea* invasion using the analyses presented in this chapter, because data are neither collected frequently enough nor do they cover a long time period. One possible solution to this is to use paleoecological techniques to supplement the monitoring record; chapter 6 uses such techniques to further investigate the ecological changes that may have contributed to the loss of *N. flexilis* in England and in the Lunan Burn lochs.

4.4 Conclusions

Examination of the existing historical botanical records, bought up-to-date with the addition of the results of SCM surveys, reveals that, since 2007, *N. flexilis* has been confirmed absent at 32 sites in the British Isles which formerly contained the species. The majority of these sites are in England, Ireland and on the Scottish mainland, however more recent losses have occurred in the Western Isles. Sites that typically contain *N. flexilis* today are circumneutral and mesotrophic. Within this bracket, *N. flexilis* is found in a variety of different conditions, in association with different plant communities. These conditions range from mildly acidic, relatively nutrient poor sites with low water clarity (a result of peat-staining), to more base-rich lakes with extremely high water clarity, to acid-neutral lakes with flora representing conditions in which nutrients are relatively readily available. *N. flexilis* grows most readily at sites where pH is not too low to inhibit the plant's ability to reproduce, but not so high that the availability of dissolved CO_2 for photosynthesis is reduced. Because of its dependence on dissolved CO_2, it is more susceptible to competition in environments where bicarbonate is the predominant form of available organic carbon. This delicate balance means that *N. flexilis* is vulnerable to the multiple and combined pressures of acidification, eutrophication and competition from *Elodea* spp., with the nature and severity of the threat at any site dependent upon the resilience of the individual ecosystem to each respective pressure. Although it is possible to deduce the likely threats to *N. flexilis* from the ecological analyses presented in this chapter, contemporary data does not cover a long enough time period to provide evidence that such pressures are responsible for the UK losses of *N. flexilis* evident in the historical botanical records. Therefore, chapter 6 adopts a paleoecological approach to further investigate the ecological changes that may have contributed to the loss of *N. flexilis* at two sites that formerly contained the species.

5. The use of seed dispersal patterns to inform paleoecological reconstructions of *N. flexilis*

5.1 Introduction

This chapter explores the ways in which knowledge of seed dispersal patterns might inform paleolimnological macrofossil reconstructions of *N. flexilis.* The key questions are:

1. How do distributions of *N. flexilis* plants relate to distributions of *N. flexilis* seeds within surface sediments?
2. How do *N. flexilis* seed abundances in surface sediments relate to *N. flexilis* cover across lakes with differing abundances of *N. flexilis*?
3. What implications do these findings have for coring strategies and the interpretation of macrofossil records when reconstructing *N. flexilis* using paleolimnology?

The extent to which temporal changes in *N. flexilis* presence and abundance can be represented in sediment cores will be investigated by examining the contemporary spatial distributions of *N. flexilis* plants and seeds at three lakes: one lake at which *N. flexilis* is abundant, one at which it is occasional and one at which it is extinct.

5.2 Results

N. flexilis plants were found at 44% of sample points at Glenastle Loch, 13% at Loch Tangy and at none of the sample points at Loch of Butterstone, the latter in keeping with the absence of *N. flexilis* in surveys since 2004. Seeds were found in the surface sediments of 97% of sample points at Glenastle Loch, 83% at Tangy Loch and 50% at Loch of Butterstone. Plant and seed distributions at each site are shown in Fig. 5.1. At Glenastle Loch, plants were primarily distributed in shallower areas of the lake, but there was no similar pattern in the distribution of seeds within the surface sediment. At Tangy Loch, *N. flexilis* plants were found primarily in the north and west margins of the site,

while seeds were found in surface sediments from across the entire lake area. As for Glenastle Loch, the distribution of seeds bore little resemblance to the distribution of the plants, with seed numbers highest towards the eastern end of the lake. At Loch of Butterstone, where *N. flexilis* were not found in the macrophyte survey, seeds were found across the entire area of the lake with a higher number of seeds found in the western half of the site. In a Kendall's tau rank correlation test, no correlation was found between *N. flexilis* percentage cover and the number of *N. flexilis* seeds in the surface sediments within individual lakes (Glenastle Loch $\tau = -0.0787$, $P = 0.21$; Tangy Loch $\tau = -0.0405$, $P = 0.64$).

The three Glew cores taken from Loch of Butterstone all show a general increase in the number of *N. flexilis* seeds with increasing sediment depth (Fig 5.2). No seeds were found in the uppermost 2cm of sediment of any of the cores. Core BUTT10 contained *N. flexilis* seeds at a sediment depth of 3cm.

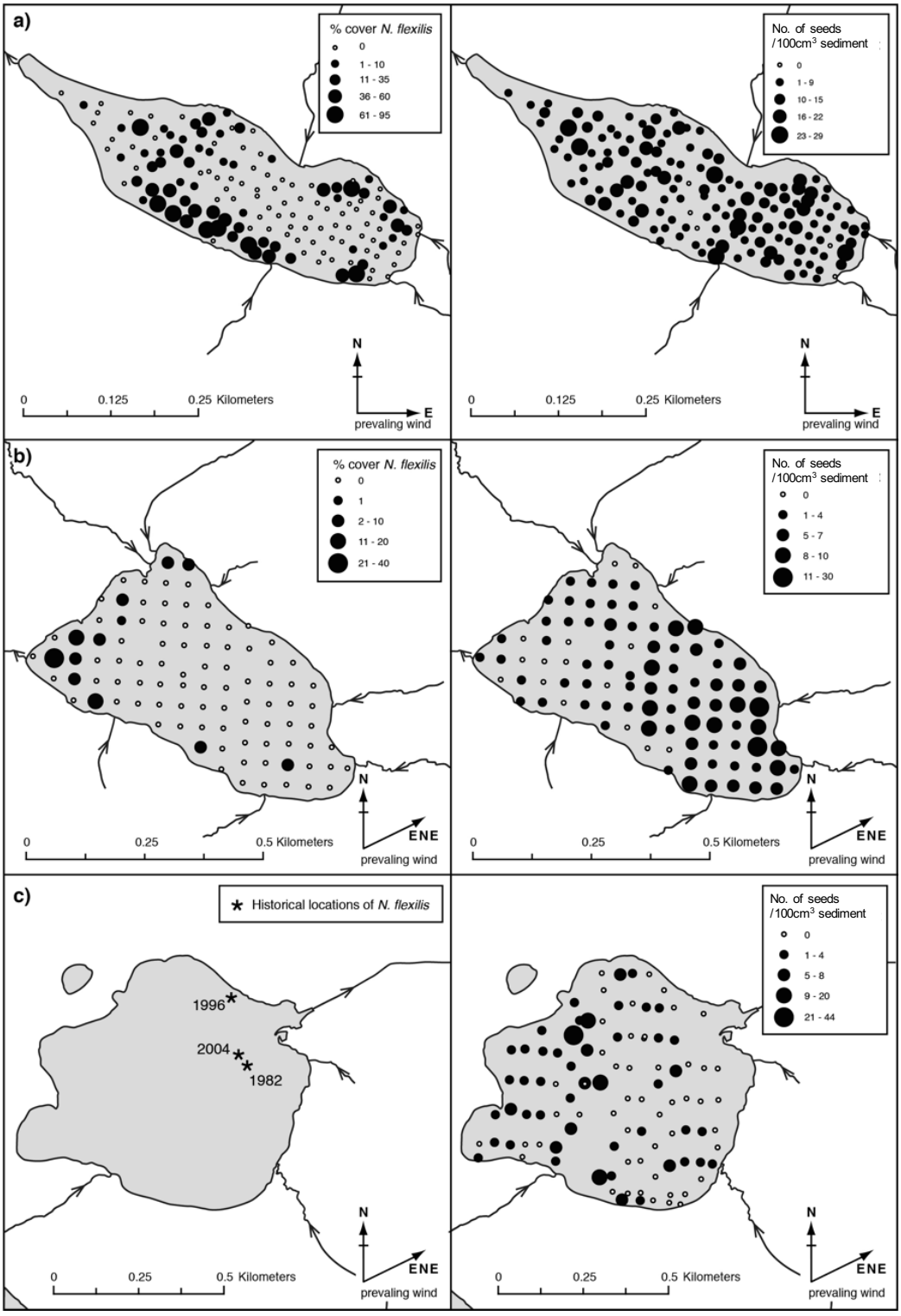

Figure 5.1 Distributions of N. flexilis *plants and seeds at a) Glenastle Loch and b) Tangy Loch, and c) historical locations of* N. flexilis *plant stands and contemporary distributions of* N. flexilis *seeds and at Loch of Butterstone.*

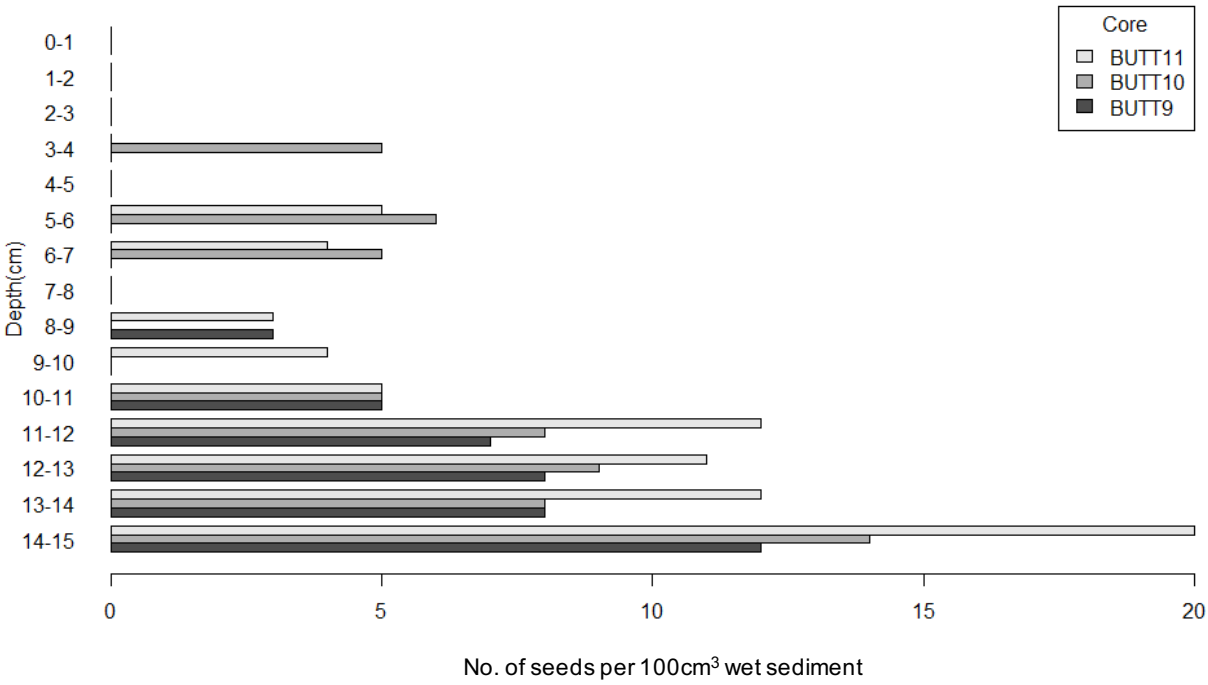

Figure 5.2 N. flexilis *seeds present in cores taken from Loch of Butterstone*

Fig. 5.3 shows scatterplots comparing the number of *N. flexilis* seeds to *N. flexilis* plant percentage cover, total macrophyte cover excluding *N. flexilis,* water depth and latitude and longitude at Glenastle Loch, Tangy Loch and Loch of Butterstone. For most variables, no correlation was found with the number of *N. flexilis* seeds. At Glenastle Loch it appears that large numbers of seeds were found at depths of 1-2m, however this is likely because the majority of sample points at this site were at these depths. There was, however, a significant correlation between seed counts and both latitude and longitude at Tangy Loch ($\tau = 0.4726$, $P = 1.64 \times 10^{-10}$ and $\tau = -0.2590$, $P = 0.001$ respectively); this suggests that seeds are clustered together spatially, in this case towards the south-east of the loch. Seed numbers were also significantly correlated with latitude at Loch of Butterstone ($\tau = -0.2311$, $P = 0.002$). There were further significant correlations between seed counts and total plant percentage cover ($\tau = -0.2017$, $P = 0.05$), and between seed counts and water depth ($\tau = 0.2017$, $P = 0.01$) at Tangy Loch.

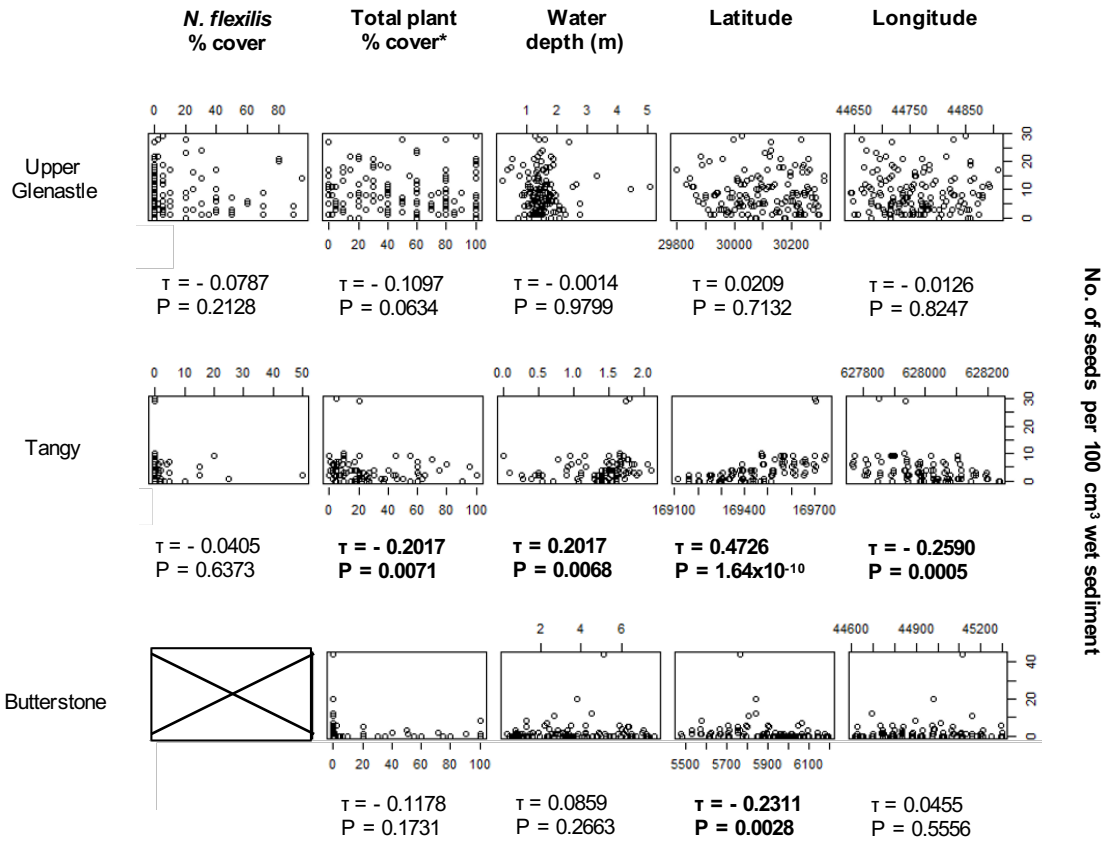

Figure 5.3 Scatterplot matrix showing influence of environmental variables on N. flexilis *seed distribution at each study site. *Total plant % cover excluding* N. flexilis

A Mann – Whitney test revealed that the means of the percentage cover of *N. flexilis* found at Glenastle Loch and Loch Tangy were significantly different from each other (z = 0.0007; P = 0.98). Table 5.1 shows that mean seed counts were highest at Glenastle Loch and lowest at Loch of Butterstone.

Table 5.1 Mean and maximum N. flexilis *seed counts from each study site. Seed counts represent number of seeds per 100cm^3.*

Site	Mean seeds per point	Maximum seeds per point
Glenastle Loch	9	29
Tangy Loch	4	30
Loch of Butterstone	2	44

Mann – Whitney tests revealed that there was a significant difference in the means of seed counts between all three sites. However, the range of seed counts was highest at Loch of Butterstone and lowest at Glenastle Loch, with the largest numbers of seeds in any sample found at Loch of Butterstone where *N. flexilis* plants were absent. Kernel density estimates were plotted for the seed counts at each of the three sites (Fig. 5.4) The intersects shown suggest that, when individual sediment grabs from all sites are considered together, seed counts below 1.37 seeds per 100cm^3 were most likely to belong to Loch of Butterstone, seed counts between 1.37 and 5.65 seeds per 100cm^3 most likely to belong to Tangy Loch, and seed counts above 5.65 per 100cm^3 most likely to belong to Glenastle Loch.

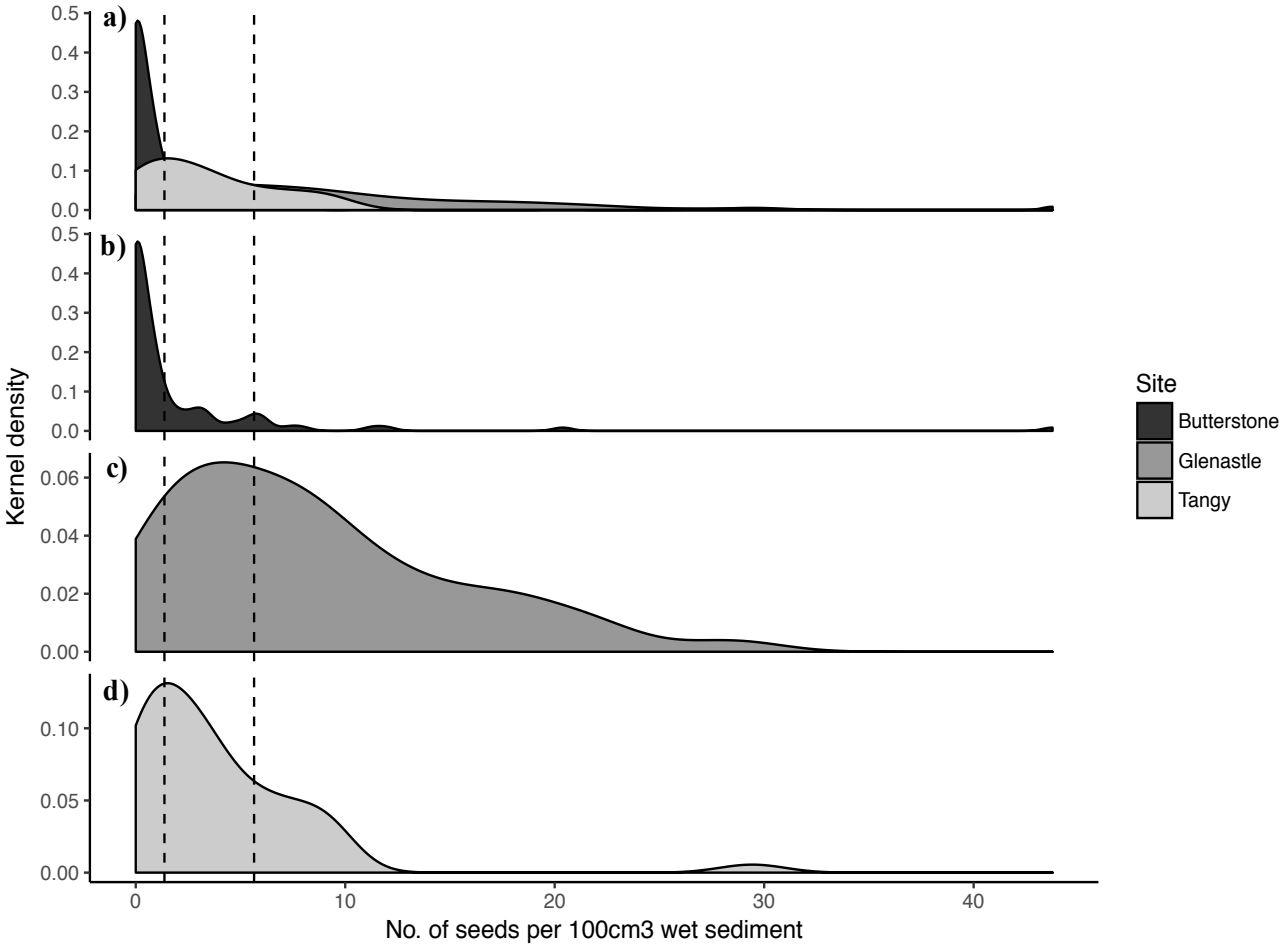

Figure 5.4 Kernel density plot showing probability distributions of seed counts at a) all study sites combined b) Loch of Butterstone c) Glenastle Loch and d) Tangy Loch. Intersections between density plot lines are marked with dashed lines. Individual samples with seed counts less than 1.37 (intersect 1) are most likely to be associated with Loch of Butterstone, whilst those with seed counts higher than 5.65 (intersect 2) are most likely to be associated with Glenastle Loch.

5.3 Discussion

5.3.1 How do *N. flexilis* seed distributions relate to distributions of parent plants?

Data from all three study lakes suggest that, irrespective of contemporary *N. flexilis*

abundance, seeds tend to be found in large numbers and are distributed across a lake basin. Seeds were also unexpectedly found in 50% of surface sediment samples at Loch of Butterstone, despite the plant being absent from the site. This accords with the findings of Birks (1973), who found *N. flexilis* macrofossils to be widespread in lakes across the environmental gradient, despite the complete absence of the plant from some sites. Furthermore, within individual lakes, there was no correlation between the numbers of seeds found in surface sediments and the percentage cover of *N. flexilis.* This implies that *N. flexilis* seeds are not concentrated close to parent plants. Seed distributions can be partially explained by plant reproductive attributes (Kolar and Lodge 2001; Levin *et al.* 2003). Many aquatic plants produce seeds that float for a limited time on water, allowing them to be carried by hydrochory (Nilsson *et al.* 2010). *N. flexilis* seeds are large, heavy, lack buoyancy and do not have an obvious adaption to dispersal. The wide distributions of *N. flexilis* seeds are therefore unexpected. It has been suggested that *N. flexilis* plants, when in seed, may either uproot or fragment and float (Preston and Croft 2001). In this study large numbers of *N. flexilis* plants were observed on the strandline of Loch Tangy, many with seeds still attached. During snorkel surveys, seeded fragments of *N. flexilis* were also observed floating on water currents just below the water surface in several lochs on the Western Isles (Fig. 5.5). This "rafting" dispersal mechanism has never been formally measured, but was hypothesised as an explanation for large numbers of *Potamogeton* seeds being found in surface sediments at Green Plantation Pond, England (Zhao *et al.* 2006). It may also explain the widespread presence of *N. flexilis* seeds in areas of lakes where no *N. flexilis* plants were found, including at Loch of Butterstone, which is hydrologically connected to two sites at which *N. flexilis* has been observed recently. If this is the case, *N. flexilis* dispersal patterns will be affected by water currents. Although there is little evidence for this being a driving force at Glenastle Loch, longitude and latitude were found to be strongly correlated with seed numbers at Loch Tangy, with the largest numbers of seeds found towards the eastern end of the loch. This end of the loch is shallower than the western end, explaining the significant correlation between seed numbers and water depth at this site. Tangy Loch is situated in a narrow valley which acts as a funnel for the wind and creates a strong prevailing westerly and thus water currents would carry *N. flexilis* rafts eastwards. At Loch of Butterstone, seeds

were clustered towards the centre and east of the loch; this is likely for similar reasons.

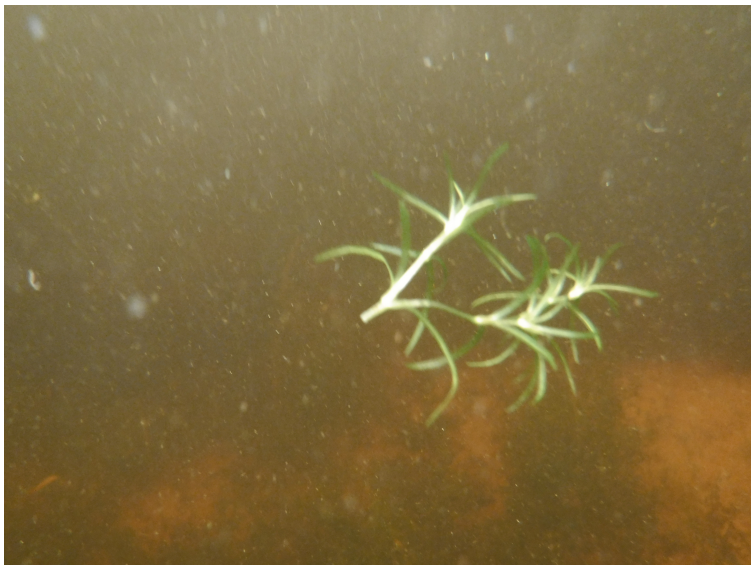

Figure 5.5 Seeded fragment of N. flexilis *floating on water currents. Photograph by author.*

An alternative explanation for the large numbers and variable distributions of seeds found in the surface sediments at Loch of Butterstone is methodological; although the Ekman grab has the advantage of being able to gather a large amount of surface sediment very quickly, it collects amalgamated sediments from the top 7 – 10cm and may therefore represent sediments laid down historically. The resolution was originally considered adequate based upon findings that *N. flexilis* seeds could germinate and grow when buried with up to 10cm of sediment (Wingfield 2002), and could therefore still present the opportunity for plant growth. Glew cores take a smaller volume of sediment, but can be extruded at much finer intervals – in this case, 1cm. The numbers of seeds in all three Glew cores from Loch of Butterstone increased with depth, and none of them contained any *N. flexilis* seeds in the upper 3cm. Based upon the sediment accumulation rates in an open water core (Bennion *et al.*, 2010), the top 3cm could represent the period from approximately 2003 to present, and the entire 15cm length of the Glew cores could represent approximately the past 100 years. *N. flexilis* was last observed at Loch of Butterstone in 2004 (Wingfield *et al.* 2006), and this aligns with the disappearance of *N. flexilis* from the Glew cores. Applying the same sedimentation rates, the Ekman grab

could collect an amalgamation of sediments laid down since ~1965 and therefore represents the historical distribution of seeds in the sediments. However, it remains clear that, at all three sites, *N. flexilis* seeds are widely distributed and that seeds are likely to be found regardless of coring location. Given that seeds were found in all three Glew cores, it is recommended that, were this study to be repeated, Glew cores be considered instead of Ekman grabs.

In a similar study at Green Plantation Pond, a small shallow English Lake, Zhao *et al.* (2006) concluded that, in general, macrofossil assemblages in sediments best represent local patch-scale vegetation within 20 – 30 m of the core site. This pattern was shown to be consistent over a period of 9 years in a follow-up study by Clarke *et al.* (2014). *Potamogeton* spp, in particular, seem to be susceptible to this (Davidson *et al.* 2005; Zhao *et al.* 2006; Salgado *et al.* 2010). However, a number of exceptions have been noted for which reproductive sub-fossils were much more widely distributed than contemporary plants. Those species with wide macrofossil distributions include *Zannichelia palustris* at Green Plantation Pond (Zhao *et al.* 2006; Clarke *et al.* 2014) and *Najas marina* and Characeae in Mediterranean lakes (Levi *et al.* 2014). All three of these species are known to produce relatively large numbers of oospores/seeds per plant and have an annual (*N. marina*) or pseudo-annual (*Z. palustris* and *Chara* spp.) reproductive strategy (Van Vierssen 1982; Bonis and Grillas 2002; Stace 2010). *N. flexilis* appears to have similar life strategies.

5.3.2 How does *N. flexilis* seed abundance relate to percentage cover of parent plants?

Although *N. flexilis* seed distributions within sites were unrelated to *N. flexilis* plant distributions, lakes with higher abundances of *N. flexilis* did harbour larger numbers of *N. flexilis* seeds than those with lower plant abundances. This may be explained by the concept of "propagule pressure". Propagule pressure refers to the number of individuals released in any one seed event and the number of discrete release events (Lockwood *et al.* 2006). Following this, plants that already have a broad distribution and/or produce large numbers of seeds are likely to disperse seeds more efficiently. At Glenastle Loch, where *N. flexilis* plants are abundant, propagule pressure is higher than at Loch of Butterstone,

where there are no *N. flexilis* plants.

5.3.3 Implications for paleolimnology

It is evident that *N. flexilis* is adapted for widespread seed dispersal. Thus, macrofossil reconstructions from sediment cores taken from any location within a basin will likely show evidence of present and past populations of *N. flexilis*. Furthermore, on average, *N. flexilis* seeds were found in higher numbers in surface sediments at sites where *N. flexilis* plants were more abundant. This suggests that, where changing trends in *N. flexilis* seed numbers in a core exist, conclusions can be drawn about the changing abundance of the plant through time. At Upton Great Broad, Norfolk, England, Ayres *et al.* (2008) successfully used historical records to confirm an increase in another member of the *Najadiceae* (*Najas marina*) that was suggested by a large increase in seed numbers towards the top of a sediment core, and the evidence presented in this study suggests that the same is possible for *N. flexilis.*

Kernel density estimations show that seed counts below 1.37 seeds per 100cm^3 wet sediment are most likely to represent very small *N. flexilis* populations, whilst those above 5.65 seeds per 100cm^3 are most likely to represent widespread, healthy *N. flexilis* populations. However, there was a very high variation in the numbers of seeds found within each site. Care must therefore be taken not to infer changes in *N. flexilis* abundance from short term fluctuations in the number of seeds found in the core. As an annual plant with an ability to disperse away from the parent plant, *N. flexilis* has the potential to change its distribution within a site on an inter-annual basis. Changing numbers of seeds in a single core where no obvious trend is present may simply represent inter-annual changes in the relative importance of factors that influence seed distribution. Further representation studies should be undertaken to expand the number of sites included to reduce the uncertainties associated with the numerical predictions presented here.

N. flexilis seeds were not only found in the surface sediments of the two sites that supported *N. flexilis,* but also at Loch of Butterstone where *N. flexilis* has not been found

since 2004. The absence of *N. flexilis* from the tops of the cores, however, suggests that the Ekman grab samples may over-estimate recent abundance of *N. flexilis*. Ekman grabs were used in this study to ensure that enough sediment was collected to allow sufficient numbers of *N. flexilis* seeds in each sample. A common problem with conventional, small bore sediment cores is that, once extruded, they do not contain enough material for macrofossil analysis to be significant (Patmore *et al.* 2014). This was a problem with the Glew cores in this study where, in most cases, a maximum of 30 – 40cm^3 wet sediment could be sieved for each sample. However, this study indicates that the Ekman grab is insufficient for further temporal work on the species and lends support to the use of wide diameter cores, such as the Big Ben sediment corer, for paleolimnological studies of *N. flexilis* and other plants (Patmore *et al.* 2014).

In accordance with the findings from Loch of Butterstone, a paleoecological investigation of Loch Flemington, Scotland, found *N. flexilis* seeds in surface sediments despite no present or past records of the plant at the site (Bennion *et al.* 2008). *N. flexilis* is notorious for being under-recorded using grapnel techniques (Capers 2000; Wingfield *et al.* 2005), and it is possible that *N. flexilis* is present in low numbers both at Loch Flemington and Loch of Butterstone. Although it is highly likely that *N. flexilis* will be recorded in a sediment core if the plant is present, the combined findings from Loch of Butterstone in this study and Loch Flemington in Bennion *et al.* (2008) mean that it cannot be assumed that the absence of *N. flexilis* seeds within a core sample represents the certain absence of *N. flexilis* plants without further investigation. Paleolimnologists should be cautious of the potential for a "false positive" when *N. flexilis* seeds are found within a sediment core at sites at which *N. flexilis* has not been recorded historically.

Several studies have shown that plant remains from different species have different distributions within lake basins (Davidson *et al.* 2005; Zhao *et al.* 2006; Salgado *et al.* 2010; Madgwick *et al.* 2011). Clarke *et al.* (2014) recommend that cores therefore be taken close enough to the shore of a lake to pick up as many remains as possible, but not so close that the sediment profile is disturbed. Others have approached the problem of patchy macrophyte distributions by taking multiple cores from lakes. For example, multiple cores taken from Barton Broad, England, demonstrated that *Chara* oospores

were historically present more frequently in the north of the lake than the south (Madgwick *et al.* 2011). Similarly, Sayer *et al.* (2010) could determine changes in spatial macrophyte distribution at Felbrigg Hall Lake, Norfolk, England, by comparing macrofossil records in five different sediment cores taken from across the site. In reconstructions of *N. flexilis* as a single species, such approaches are unnecessary since seeds are highly likely to be found in cores taken from any location within a basin.

5.4 Conclusions

N. flexilis is adapted for dispersal of seeds away from the parent plant, and seeds are distributed widely across lake basins. It is therefore an ideal subject for paleolimnological work since seeds are highly likely to be present in any wide-bore sediment core taken from a basin containing the plant. *N. flexilis* seed counts are higher on average at sites with a higher abundance of *N. flexilis* plants, and seed counts above 5.65 seeds per 100cm^3 wet sediment are most likely to represent a widespread, healthy population of *N. flexilis*. However, the largest number of seeds in a single surface sediment sample in this study was found at Loch of Butterstone, where *N. flexilis* plants were not present. *N. flexilis* seeds therefore have the potential to be over-represented in sediment cores, particularly in cases where plant numbers are low. Special care should be taken not to draw spurious conclusions on any changes in *N. flexilis* abundance that are not based upon clear, consistent trends in seed numbers within sediment cores, ideally backed up by historical records. Using macrofossils as a proxy for *N. flexilis* abundance in conjunction with information on dispersal potential may reveal information on the apparent decline of *N. flexilis* in Scotland over the past 100 years, and this is investigated further in chapter 6.

6. Paleoecological explorations of the decline of *N. flexilis* at Esthwaite Water and Loch of Craiglush

6.1 Introduction

This chapter uses paleoecology to investigate the decline of *N. flexilis* at Esthwaite Water and Loch of Craiglush (the first loch in the Dunkeld-Blairgowrie chain). The objectives are:

1. To reconstruct the changes in *N. flexilis* populations over the last ~150 years using a combination of historical botanical records and macrofossil reconstructions.
2. To use macrofossils, diatoms and cladocera as proxies for environmental change to explore the factors driving observed alterations in *N. flexilis* during the Anthropocene.

The decline of *N. flexilis* is investigated using high resolution analyses of one dated sediment core taken from the littoral zone of each of the two study sites, in combination with existing data from open water (profundal) cores from the same sites, and historical monitoring data.

6.2 Results

6.2.1 Esthwaite Water littoral core: ESTH9

Core characteristics

A 109cm long core – ESTH9 – was collected at Esthwaite Water in a water depth of 1.5m. Below 25cm, the core was grayish brown in colour (5YR 4/2 on the Munsell chart), with a gradual change to brownish black (5YR 2/2) towards the top of the core. Ferrous streaking was present in the sediments throughout. The entire core had a relatively high organic content. Loss on ignition was 24% at the bottom of the core, decreasing to approximately 18% at 37cm. It remained stable at ~18% until

approximately 25cm, where it gradually increased back to 24% at the top of the core (Fig. 6.1). Carbonate levels were stable at 3-5% throughout the core, except for the top ~10cm, where they declined to ~2%.

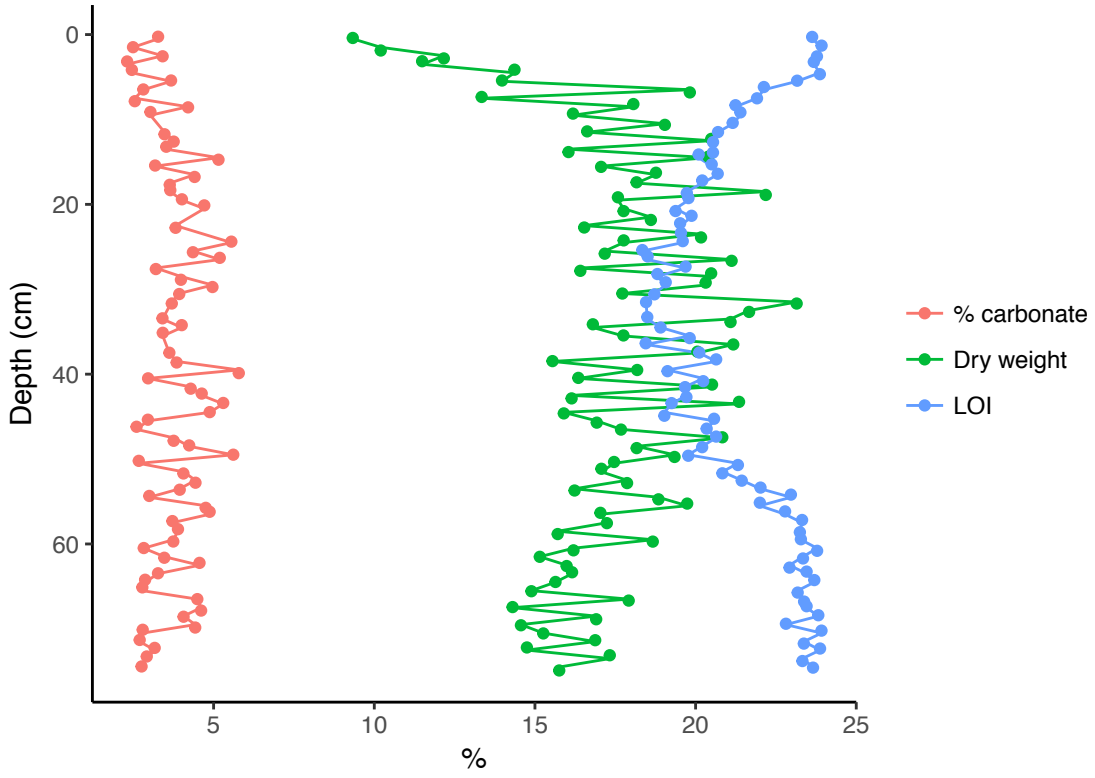

Figure 6.1 Changes in % dry weight, % loss on ignition and % carbonate in ESTH9

Core chronology

Equilibrium of total ^{210}Pb activity with supported ^{210}Pb activity is reached at ~26cm (Fig 6.2a). The maximum value of unsupported ^{210}Pb activity is at ~3.5cm, suggesting increased sediment accumulation towards the top of the core (Fig. 6.2b). Between 9.5 and 15.5cm, unsupported ^{210}Pb activities decline exponentially with depth, implying stable sedimentation rates within this section. However, a sharp dip at 17.5cm suggests a possible sediment slumping event. The CRS model places 1986 at 7.5 – 9.5cm. This is in agreement with the ^{137}Cs peak at 9.5cm, which likely represents the 1986 fallout from

Chernobyl (Fig. 6.2c). A relatively slow gradual increase in ^{137}Cs activity from the deep side of the ^{137}Cs peak, combined with the detectable ^{241}Am from 9.5cm – 13.5cm, suggests that the ^{137}Cs peak expected from the 1963 peak testing of nuclear weapons is obscured by the fallout from the Chernobyl accident. The CRS model places 1963 between 11.5 – 13.5cm. The full chronology is shown in Table 6.1. Sedimentation rates before the 1920s were calculated at ~0.02 – 0.03g cm^{-2} yr^{-1}. In the 1930s, the sedimentation rate peaks at 0.06g cm^{-2} yr^{-1}, before recovering until another increase in the 1970s. Over the last 30 years, sedimentation rates have fluctuated between 0.036 and 0.057g cm^{-2} yr^{-1}.

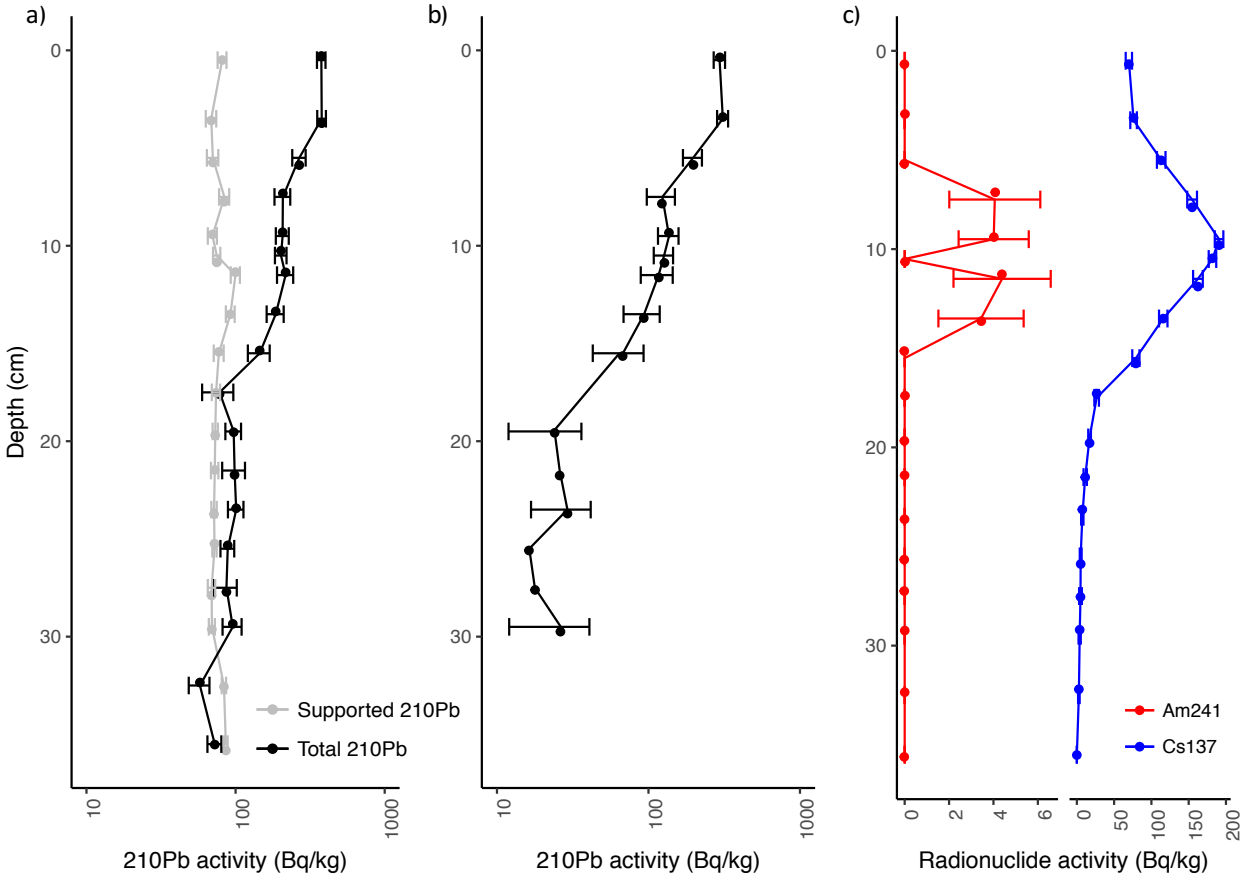

Figure 6.2 Fallout radionuclide concentrations in ESTH9: a) total ^{210}Pb; (b) unsupported ^{210}Pb; and c) ^{137}Cs and ^{241}Am.

Table 6.1 Core chronology of ESTH9.

Depth	Drymass	Chronology			Sedimentation Rate		
		Date	Age				
cm	g cm^{-2}	AD	yr	±	g cm^{-2} yr^{-1}	cm yr^{-1}	± %
0	0	2013	0				
0.5	0.0406	2012	1	2	0.0527	0.379	10.8
3.5	0.4866	2002	11	2	0.0367	0.24	11.8
5.5	0.8052	1994	19	2	0.0451	0.257	17.4
7.5	1.189	1987	26	3	0.0567	0.291	24
9.5	1.5858	1978	35	4	0.0395	0.203	21.4
10.5	1.7711	1973	40	5	0.0365	0.191	22.4
11.5	1.9678	1968	45	6	0.0333	0.181	30.7
13.5	2.3236	1956	57	8	0.0292	0.166	37.1
15.5	2.6726	1944	69	11	0.0274	0.152	50.4
17.5	3.0425	1938	75	12	0.0607	0.318	61.3
19.5	3.4352	1936	77	12	0.0608	0.302	63.9
21.5	3.848	1928	85	15	0.0439	0.204	82.2
23.5	4.2974	1915	98	21	0.0258	0.119	79
25.5	4.7148	1900	113	25	0.0285	0.145	86.1
27.5	5.086	1882	131	28	0.0151	0.081	91.5

N. flexilis*: Macrofossils and historical records*

Large numbers of *N. flexilis* seeds (up to 207 per $100cm^3$ wet sediment) were found between 24 and 35cm (i.e. pre-1915) (Fig. 6.3). Smaller numbers of seeds (up to 7 per $100cm^3$ wet sediment) were found between 24 and 9cm (1915 – 1978). Above 9cm, *N. flexilis* seeds disappeared, except for at 3.5cm (2002), where 4 seeds per $100cm^3$ wet sediment were found. *N. flexilis* leaf spines were found in highest abundance below 21cm (pre-1928). Historical records indicating the presence of *N. flexilis* between 1914 and 1982 broadly correspond with the presence of *N. flexilis* remains in the core. Based upon

the numbers of seeds found in the core, the results presented in chapter 5, and the historical records, the core was split into three zones based upon *N. flexilis* abundance to facilitate description and interpretation: High *N. flexilis* abundance below 24cm (pre-1915); low *N. flexilis* abundance between 24 and 9cm (1915 – 1982); *N. flexilis* absence (1982 – present).

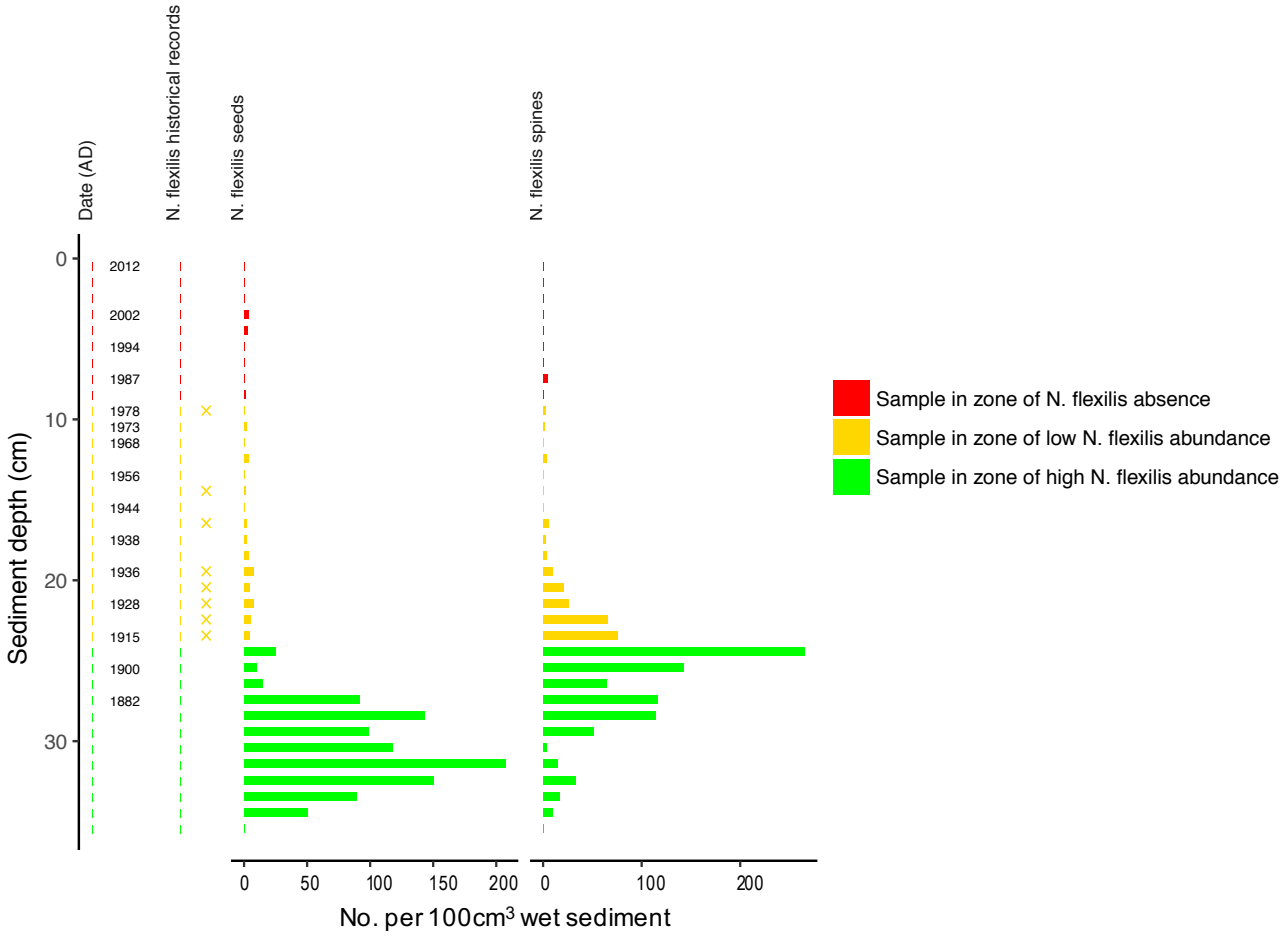

Figure 6.3 Changes in numbers of N. flexilis seeds and leaf spines in ESTH9, plotted alongside historical botanical records.

Macrofossils

Changes in floral macrofossil remains were evident, and are shown in comparison to the identified *N. flexilis* zones in Fig. 6.4. Fig. 6.5 shows the first two axes of a Principal

Components Analysis of the composition of the samples. The first three axes of this PCA explain 72.33% of the variance in the composition of the samples in ESTH9. Axis one, which explains 46.42% of the variance, is strongly correlated with *N. flexilis* seeds and spines. This suggests that *N. flexilis* abundance is strongly linked to floristic composition. Clustering of samples from each of the *N. flexilis* abundance zones within the PCA, further implies differences in the floristic community of samples linked to *N. flexilis* abundance. In the zone of high *N. flexilis* abundance, and extending up to ~20cm (1938), samples were also associated with *Chara* spp., *Nitella* spp., and moss remains. Samples from the zone of low *N. flexilis* abundance were associated with a high diversity of plant remains, including various *Potamogeton* species, *Isoetes lacustris* and *Utricularia vulgaris*. Samples from the zone where *N. flexilis* was absent were associated with high numbers of *Nymphaea* triclosclerids and *Typha latifola* remains.

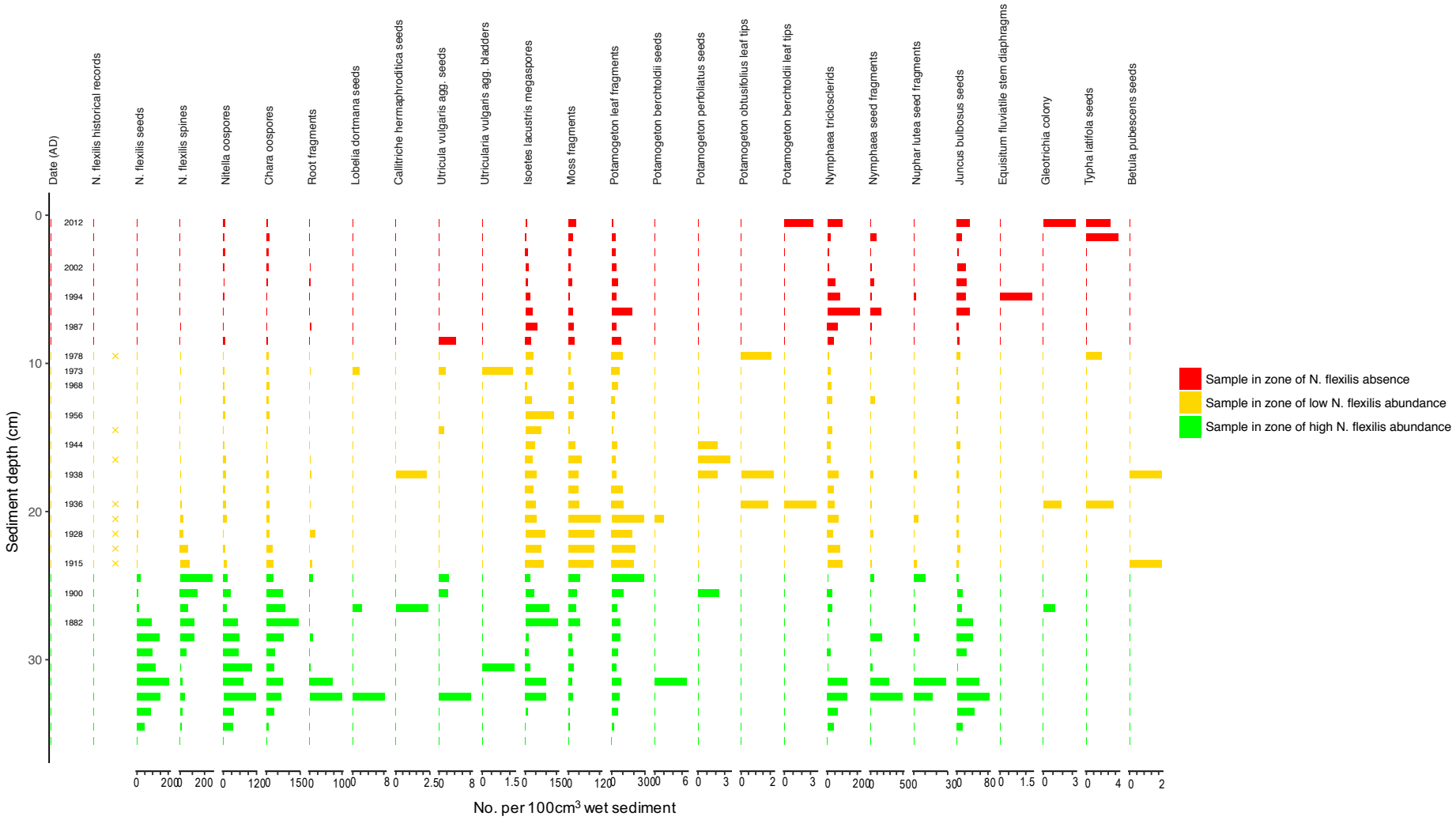

Figure 6.4 Changes in plant macrofossil numbers through time in ESTH9, colour-coded by N. Flexilis abundance and plotted alongside historical botanical records for N. flexilis.

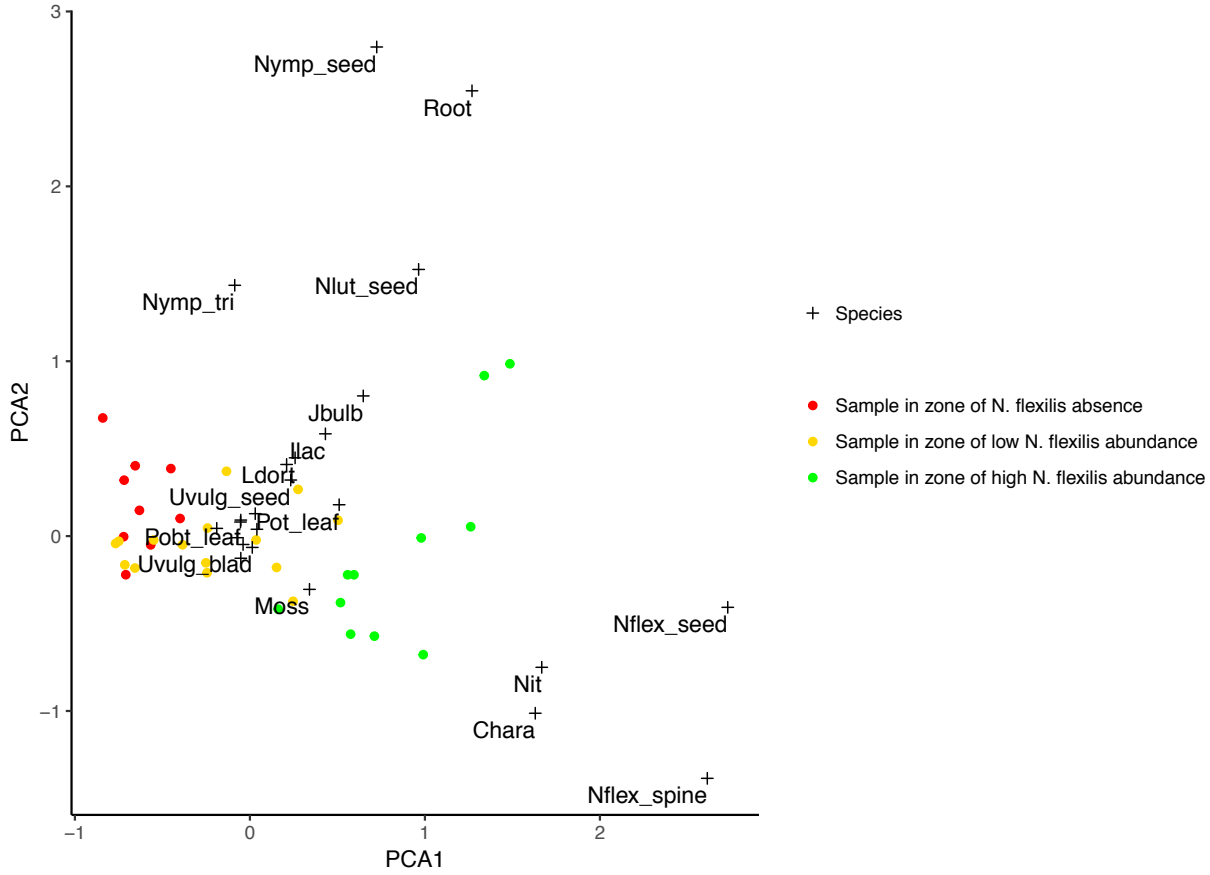

Figure 6.5 Principal Components Analysis of plant macrofossils in ESTH9, colour-coded by N. flexilis *abundance. Plotted axes explain 64% of variance. Species abbreviations are defined in Appendix 7.*

Changes were also evident in the faunal macrofossil remains (Fig. 6.6), although Principal Components Analysis (Fig. 6.7) suggests that these changes were limited to a few species. The first axis of the PCA explains 33.99% of the variance, and is negatively correlated with *Crystatella* statoblasts and *Trichoptera* pupal cases and positively correlated with *Daphnia* ephippia, and the second axis explains 23.75% of the variance in the data and is correlated with *Chidoridae* carapaces. Whilst the largest numbers of *Crystatella* statoblasts coincide with the zone of high *N. flexilis* abundance, a decline in *Trichoptera* remains is gradual and extends to ~18cm (1938) - well into the zone of low *N. flexilis* abundance. An increase in *Daphnia* ephippia also occurs in this zone at ~12cm (1968), before the disappearance of *N. flexilis.* Nevertheless, samples with similar *N.*

flexilis abundances were grouped together in the PCA plot. Samples with high *N. flexilis* abundance were associated with high numbers of *Crystatella* statoblasts and *Trichopetera* pupal cases, whilst samples where *N. flexilis* was absent were associated with *Daphnia* ephippia.

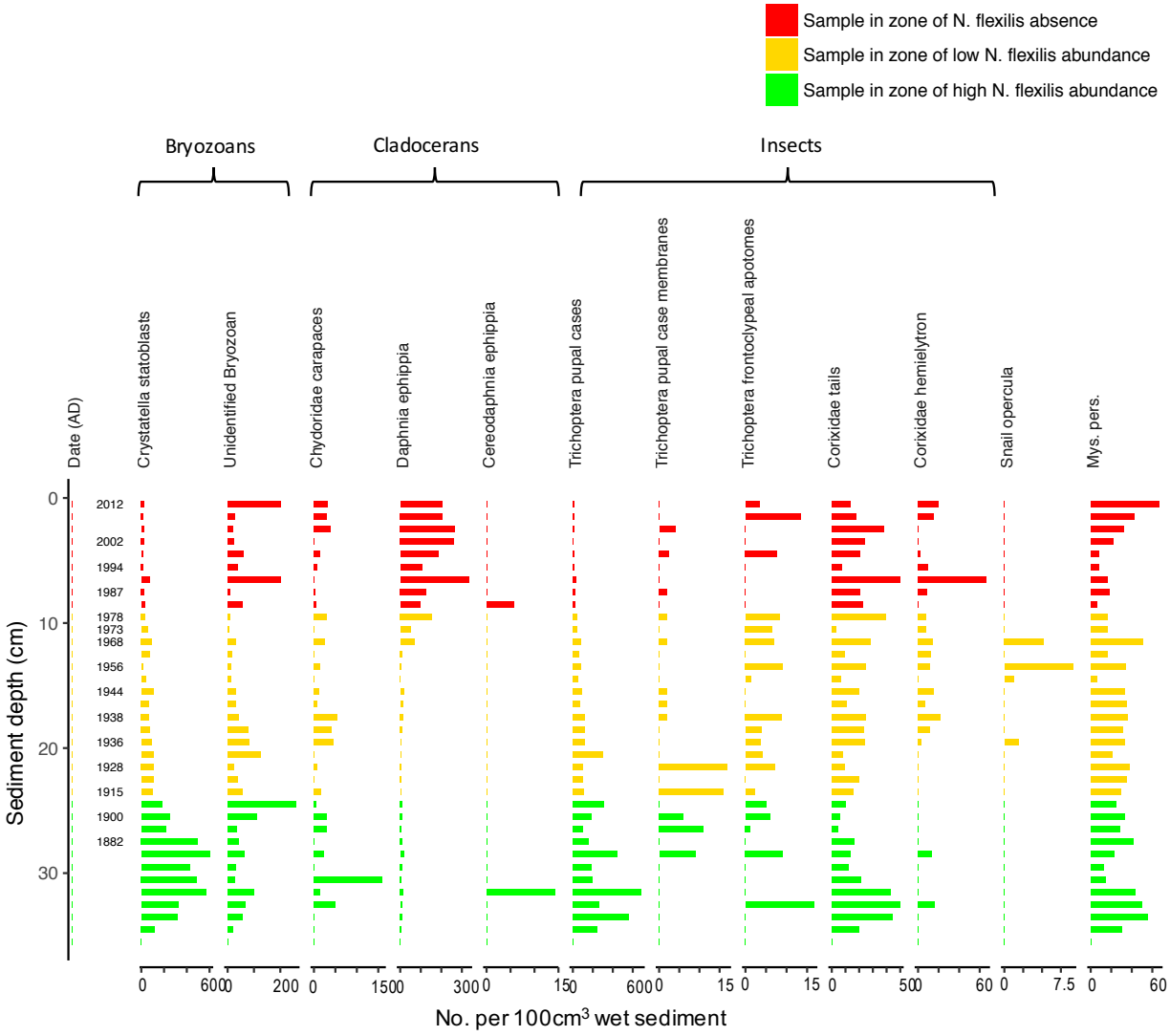

Figure 6.6 Changes in faunal macrofossil numbers through time in ESTH9, colour-coded by associated N. flexilis abundance.

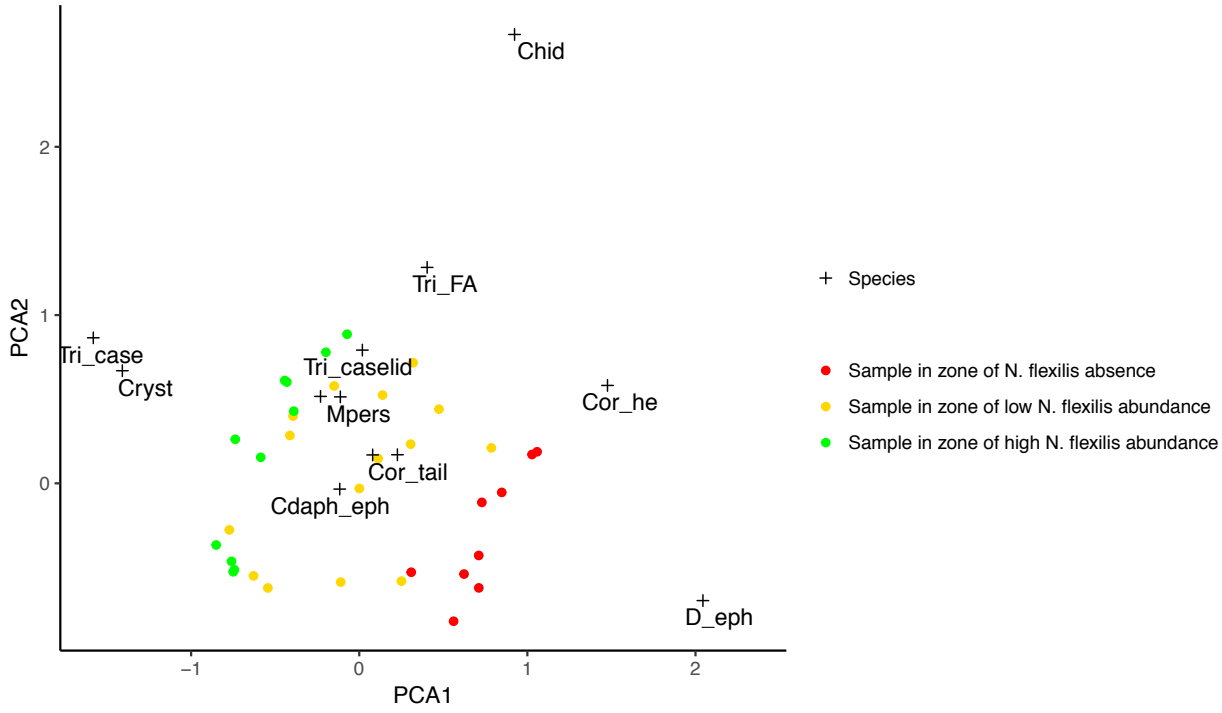

Figure 6.7 Principal Components Analysis of faunal macrofossils in ESTH9, colour-coded by associated N. flexilis *abundance. Plotted axes explain 58% of variance. Species abbreviations are defined in Appendix 7*

Chitinous Cladocera remains

Unlike the macrofossil remains, changes in the subfossil Cladocera remains do not align with changes in *N. flexilis* abundance. Fig. 6.8 shows that the main community shift occurred at ~19cm (1936), towards the middle of the zone of low *N. flexilis* abundance. Below ~19cm, the total number of Cladocera remains in each sample was larger than above ~19cm. Samples in the lower part of the core contained larger percentages of *Bosmina longispina* and *Camptocercus rectirostris.* Samples above ~19cm were associated with larger percentages of *Bosmina longisrostris, Alona affinis, Alona quadrangularis* and *Chydorus sphericus.* The first three axes of a PCA explain 73.44% of

the variance in cladocera species composition in the core (Fig. 6.9). Because of the timing of the community change, samples with similar abundances of *N. flexilis* are not grouped as clearly as for the macrofossil data. However, samples with high *N. flexilis* abundance are generally associated with higher relative abundances of *Bosmina longispina, Camptocercus rectirostris* and *Sida crystallina.*

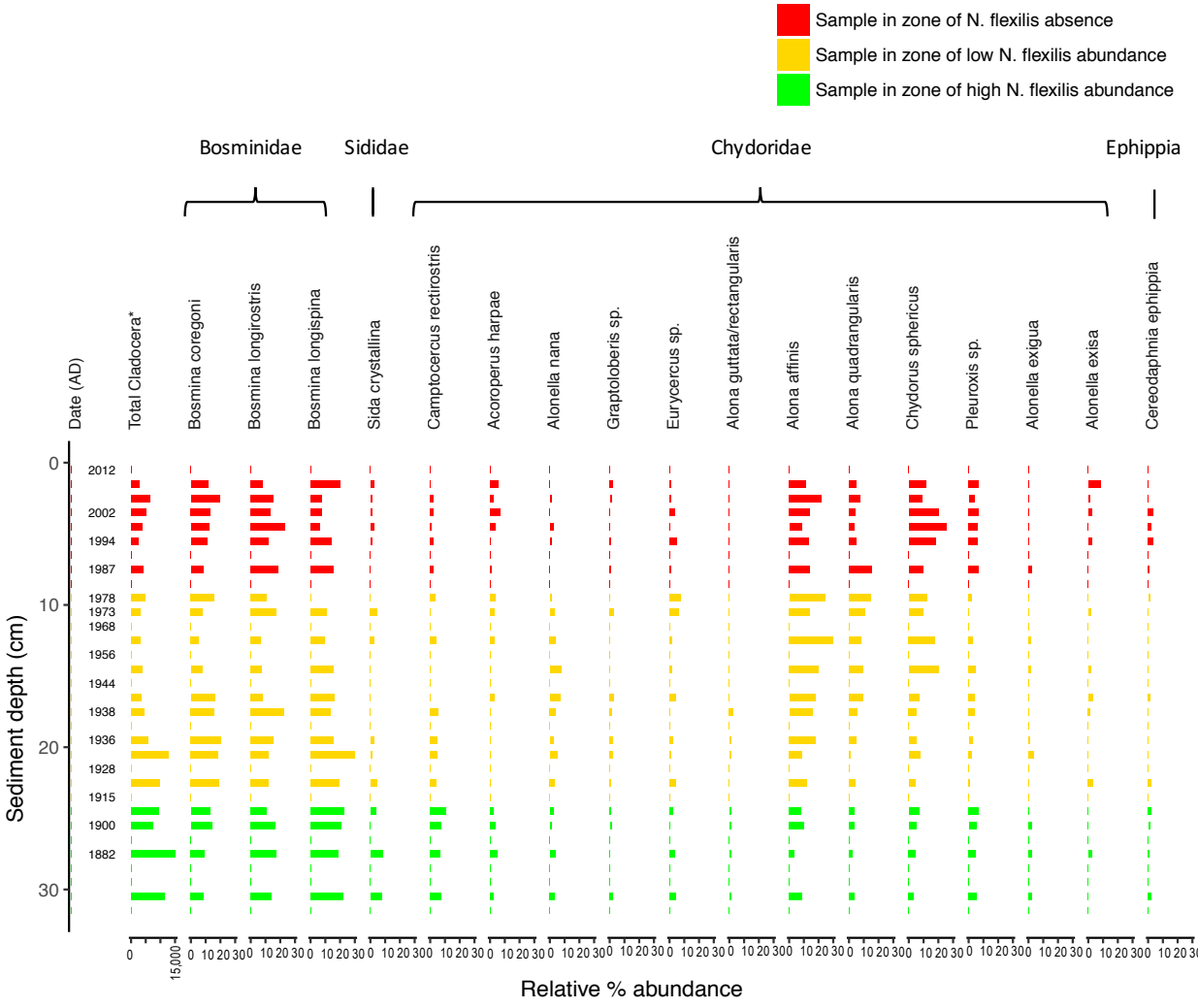

*Figure 6.8 Changes in relative percentage abundance of Cladocera species through time in ESTH9, colour-coded by associated N. flexilis abundance. * x-axis for total Cladocera shows absolute counts.*

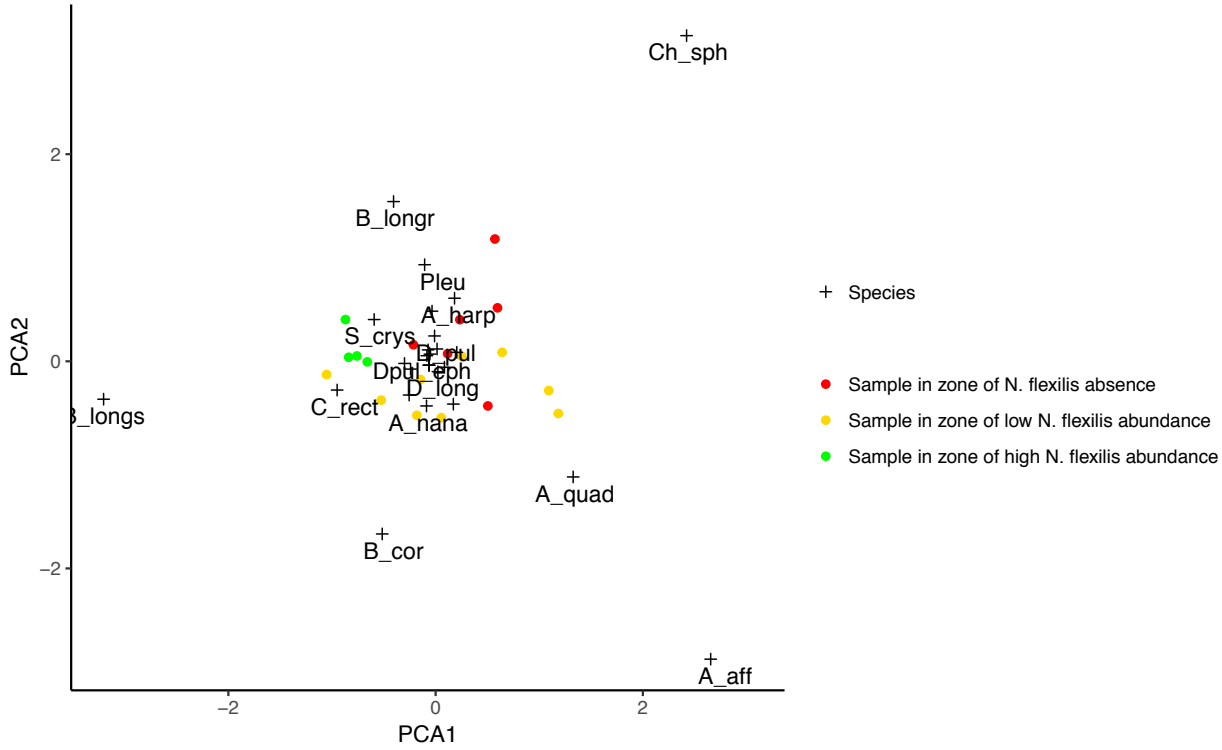

Figure 6.9 Principal Components Analysis of Cladocera species in ESTH9, colour-coded by associated N. flexilis *abundance. Plotted axes explain 65% of variance. Species abbreviations are defined in Appendix 7.*

6.2.2: Esthwaite Water pelagic core: ESTH7

A full description of ESTH7 and its chronology is given by Dong *et al.* (2012). The most notable feature of the core is a sediment slumping event at ~27cm. The chronological uncertainties produced by this slumping event were resolved by Dong *et al.* (2012) by combining ESTH7 with another pelagic core: ESTH1. The characteristics and chronology of ESTH1 is described in Bennion *et al.* (2000). A total of 112 diatom taxa were identified by Dong *et al.* (2012) in the combined core, which extended to ~750 AD. 82.41% of the variance between diatom assemblages of samples in the post ~1880 section

of the combined core is explained in the first three axes of a PCA (Fig. 6.11). Most of the variation is explained by differences in a few key species: *Achnanthes minutissuma, Tabellaria flocculosa var. flocculosa, Cyclotella comensis, Cyclotella radiosa, Asterionella formosa, Aulacoseira subarctica, Fragilaria crotenensis* and *Stephanodiscus hantzschii.* Samples from dates representing *N. flexilis* presence and *N. flexilis* absence were clustered together, implying that the disappearance of *N. flexilis* was associated with changes in the diatom community. Two major floristic zones were identified by Dong *et al.* (2011) and Dong *et al.* (2012) in this section of the core; one from ~1880 to ~1976, and one from ~1976 to the top of the core (2006). Fig. 6.10 shows that the timing of this major change precedes the disappearance of *N. flexilis* from the lake by ~5 years, but there are some changes in individual species relative abundances that correlate more closely with changes in *N. flexilis* abundance. In the PCA, samples from the time zone where *N. flexilis* abundance was high were associated with *Achnanthes minutissima* and *Cyclotella comensis;* the latter declines in abundance at the same time as *N. flexilis. Fragilaria crotonensis,* which is associated with *N. flexilis* absence in the PCA, first appears at the same time as *N. flexilis* abundance decreases in ~1915. *Tabellaria flocculosa* reaches its maximum abundance during the zone of low *N. flexilis* abundance. From ~1976, *Achnanthes minutissima, Tabellaria flocullosa, Cyclotella comensis* and *Cyclotella radiosa,* which were relatively abundant when *N. flexilis* was present, decline, and *Fragilaria crotonensis, Stephanodiscus hanzchii* and several *Aulacoseira* species appear in the record. These changes become more pronounced throughout the period of *N. flexilis* absence.

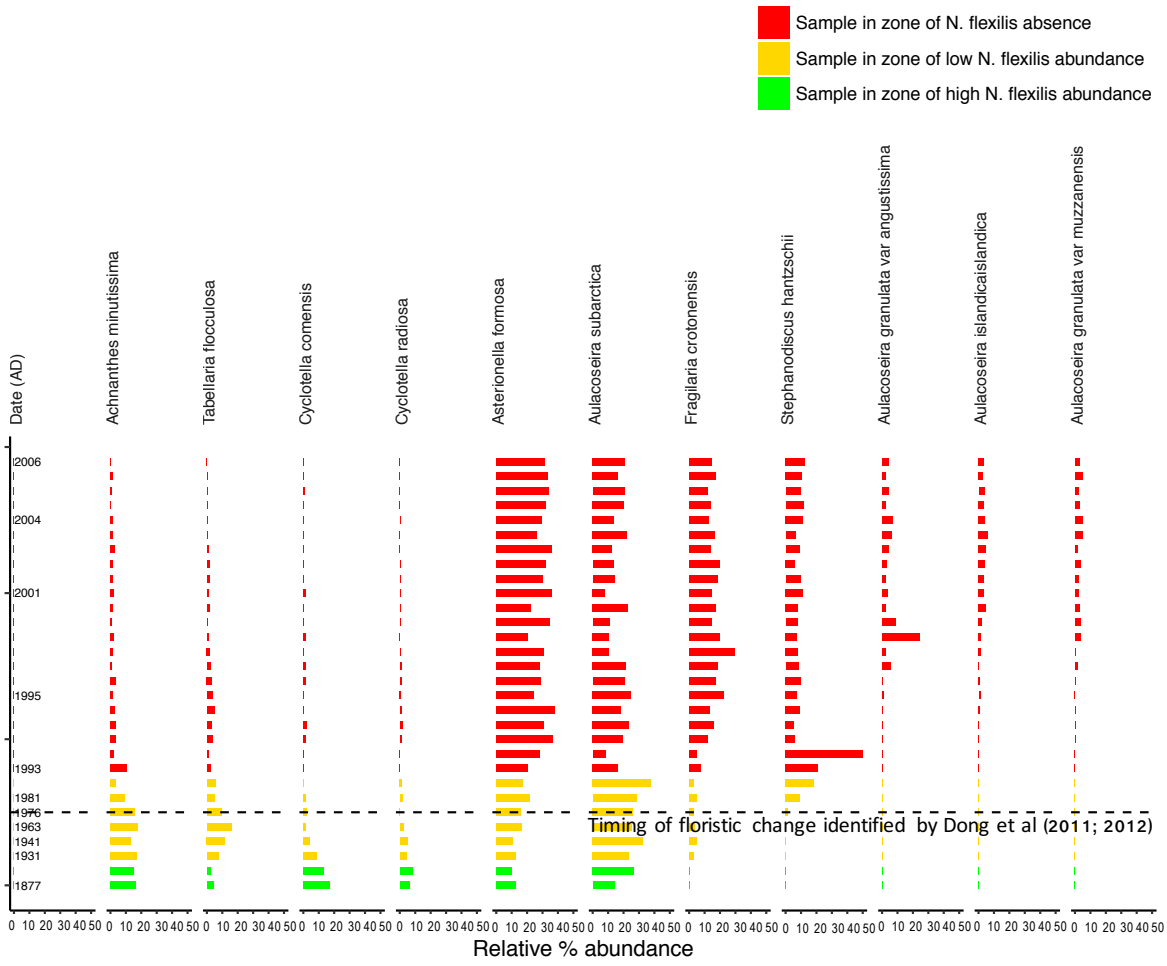

Figure 6.10 Changes in relative percentage abundance of major diatom species through time in combined ESTH1/ESTH7 core, colour-coded by associated N. flexilis abundance. Not all species shown - a full list can be found in Appendix 7.

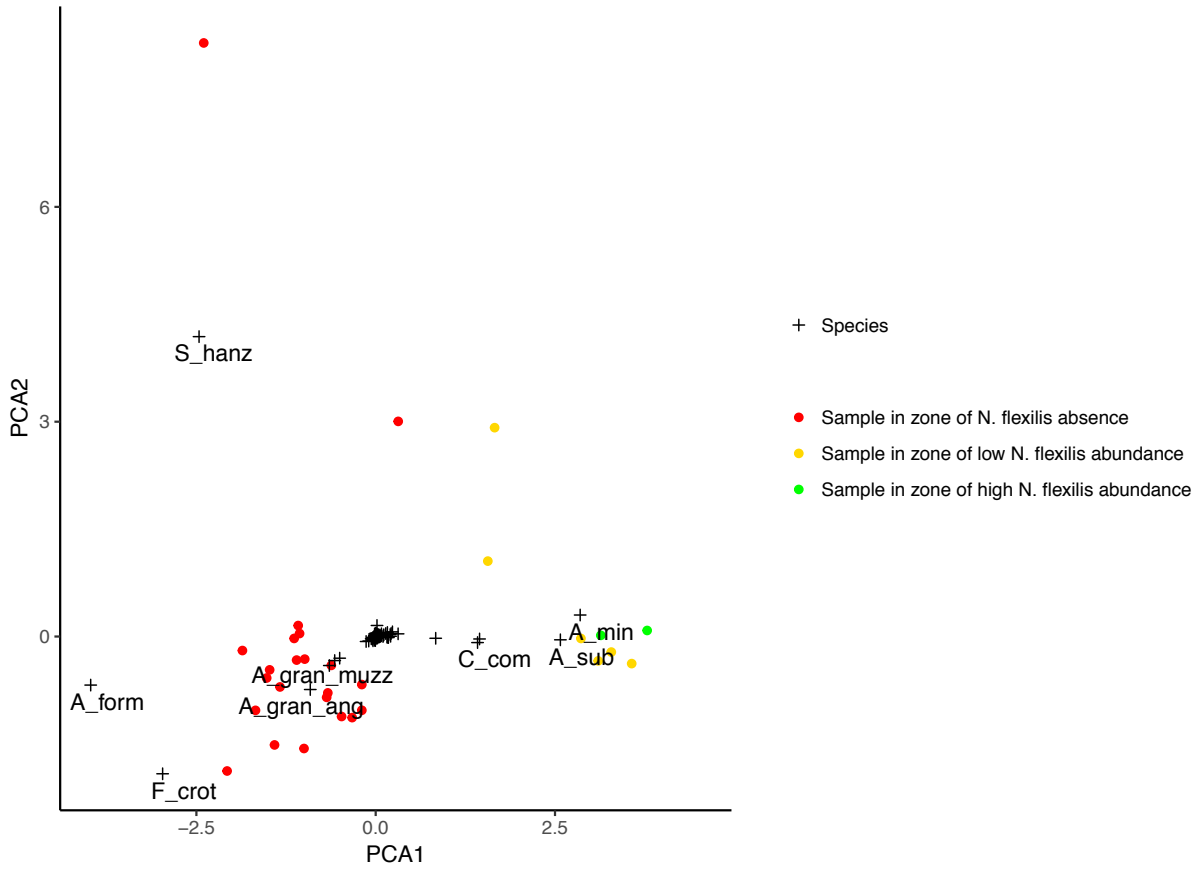

Figure 6.11 Principal Components Analysis of diatom species in combined ESTH1/ESTH7 core, colour-coded by associated N. flexilis *abundance. Plotted axes explain 65% of variance. Species abbreviations are defined in Appendix 7.*

6.2.3 Esthwaite Water monitoring data

Water chemistry monitoring data from Esthwaite Water since 1945 is shown in Fig. 6.12, in comparison to reconstructed *N. flexilis* abundance. Alkalinity, nitrates, phosphates, soluble reactive phosphorus and water temperature all show increasing trends and increasing interannual variability since 1945. Secchi depth trends are the inverse, and have decreased from a maximum of ~4m to a minimum of ~2m. over time. The loss of *N. flexilis* in 1982 was immediately preceded by rapid rates of change in alkalinity, total phosphorus, phosphates and nitrates, as well as being associated with reduced Secchi depth. Since ~2000, recovery to levels recorded preceding the loss of *N. flexilis* has been recorded in all measures of nutrient concentrations. Secchi depth has also started to

recover, but remains lower than its 1982 average. Alkalinity and surface water temperature continue to increase.

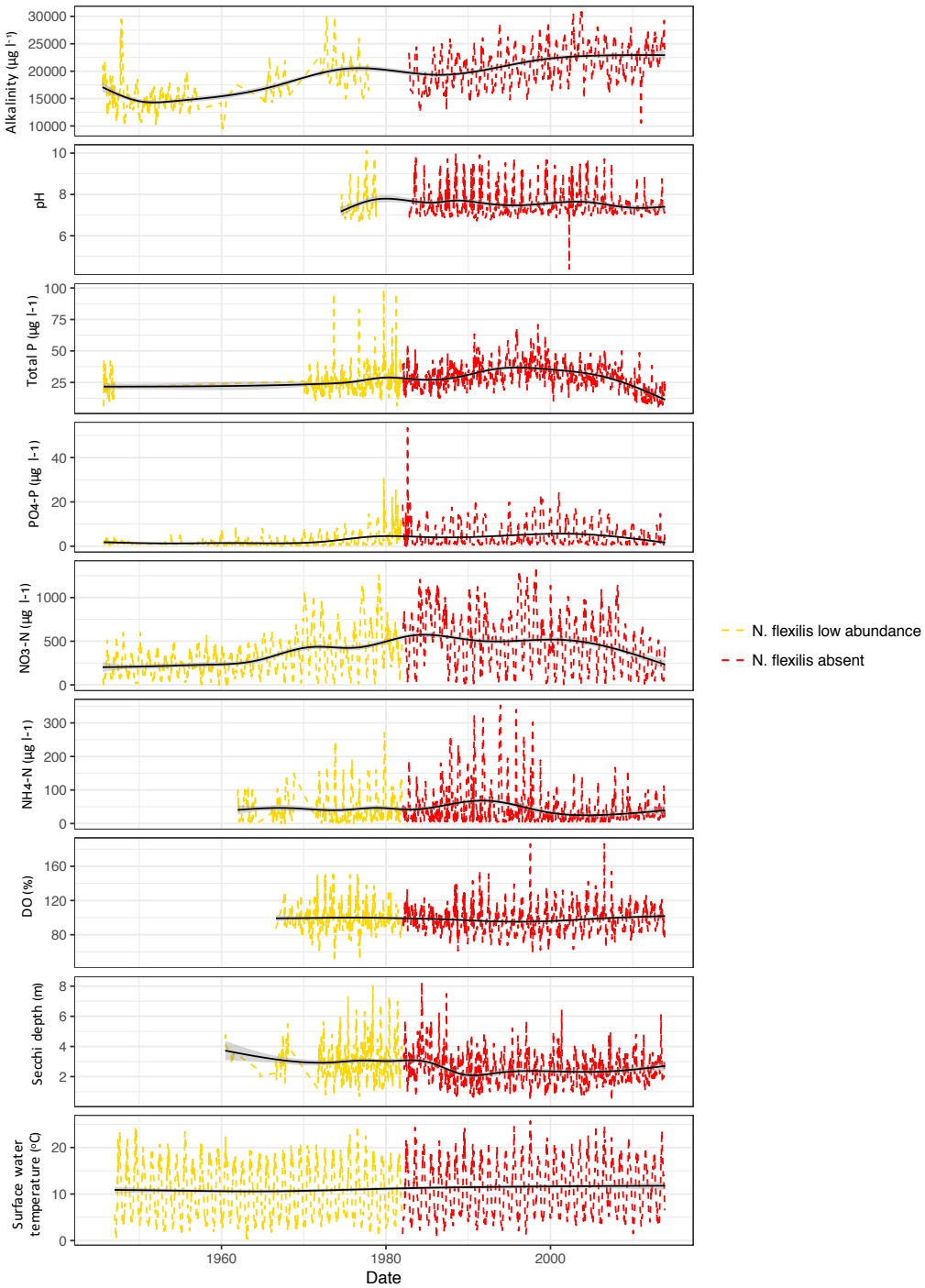

Figure 6.12 Water chemistry monitoring data from Esthwaite Water between 1945 and 2014, with GAM smoothing (black line) to visualize trends. Records are colour-coded according to associated N. flexilis *abundance.*

Fig. 6.13 (overleaf) shows the aquatic macrophyte species recorded during historical surveys at Esthwaite Water between 1917 and 2014. Whilst there is some overlap between species that were recorded before and after *N. flexilis* disappeared in 1982, it is clear that *N. flexilis* is not the only plant to have been lost from the site. *Hydrilla verticillata var. pomeranica, Potamogeton alpinus* and *Potamogeton perfoliatus* have not been recorded since 1917, and *Potamogeton gramineus, Ranunculus peltatus* and *Lobelia dortmana* have not been recorded since 1980. Several plant species have appeared in the record since *N. flexilis* disappeared, including *Caltha palustris, Ceratophyllum demersum, Elodea nuttallii, Phragmites australis, Schoenoplectus lacustris* and *Typha latifola.*

6.2.4 Loch of Craiglush littoral core: CRAIB

Core characteristics

A 56cm long core – CRAIB – was collected at Loch of Craiglush in a water depth of 1.1m. The bottom half of the core was composed of reddish black sediments (10YR 2/1 on the Munsell chart), with a slow transition to dark grayish red (10YR 3/2), upwards of 20cm. Changes in % dry weight, % LOI and % carbonate are shown in Fig. 6.14. Organic content was high (>20% LOI) throughout the core, but peaked at ~40% once at about ~50cm and once at the top of the core. Carbonates were low (2 – 5%) throughout the core.

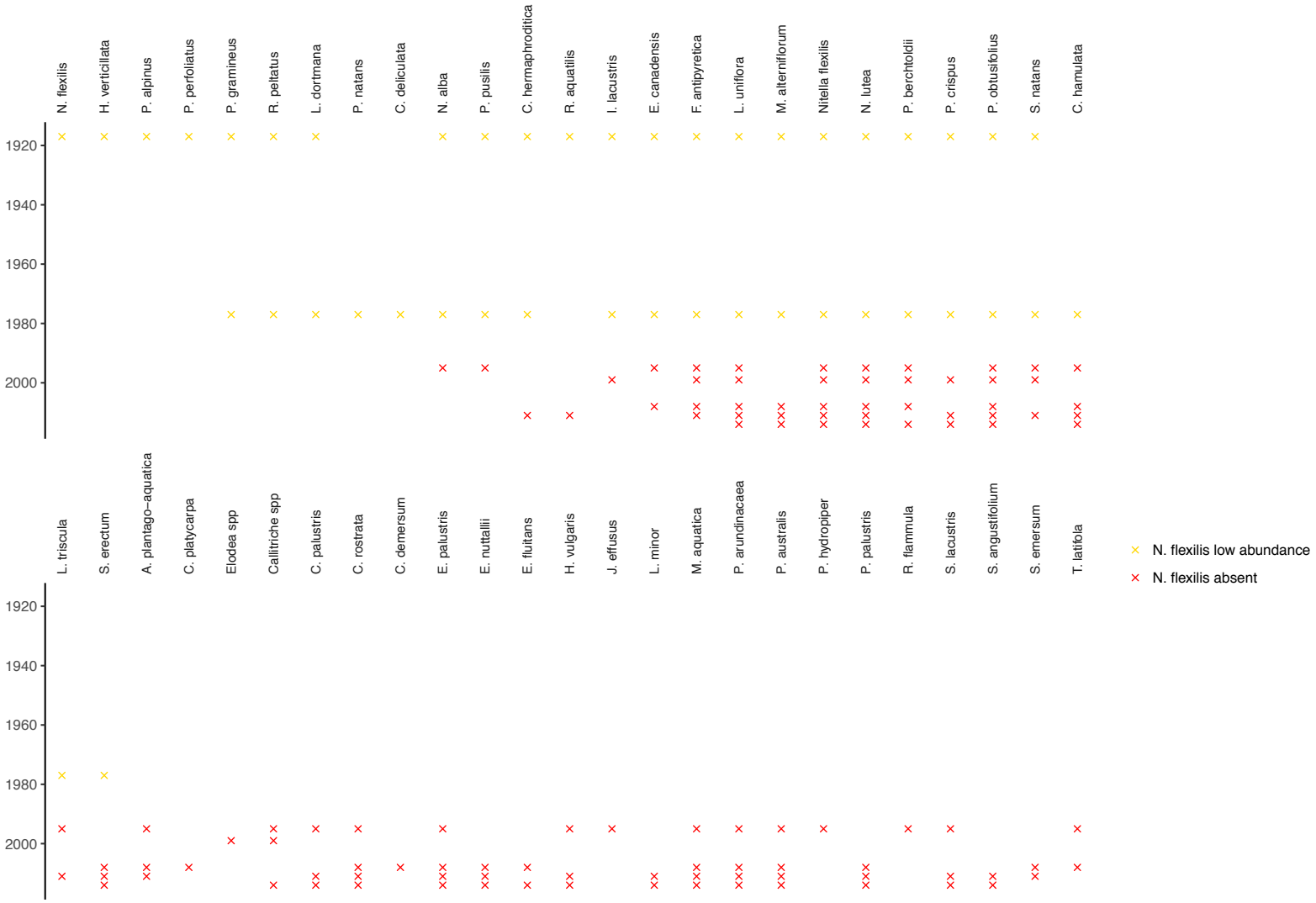

Figure 6.13 Historical macrophyte records from Esthwaite Water. Sources: Pearsall (1917), Stokoe (1983), Bennion et al (2000), Darwell (2000), and unpublished SCM data (2008, 2011 and 2014).

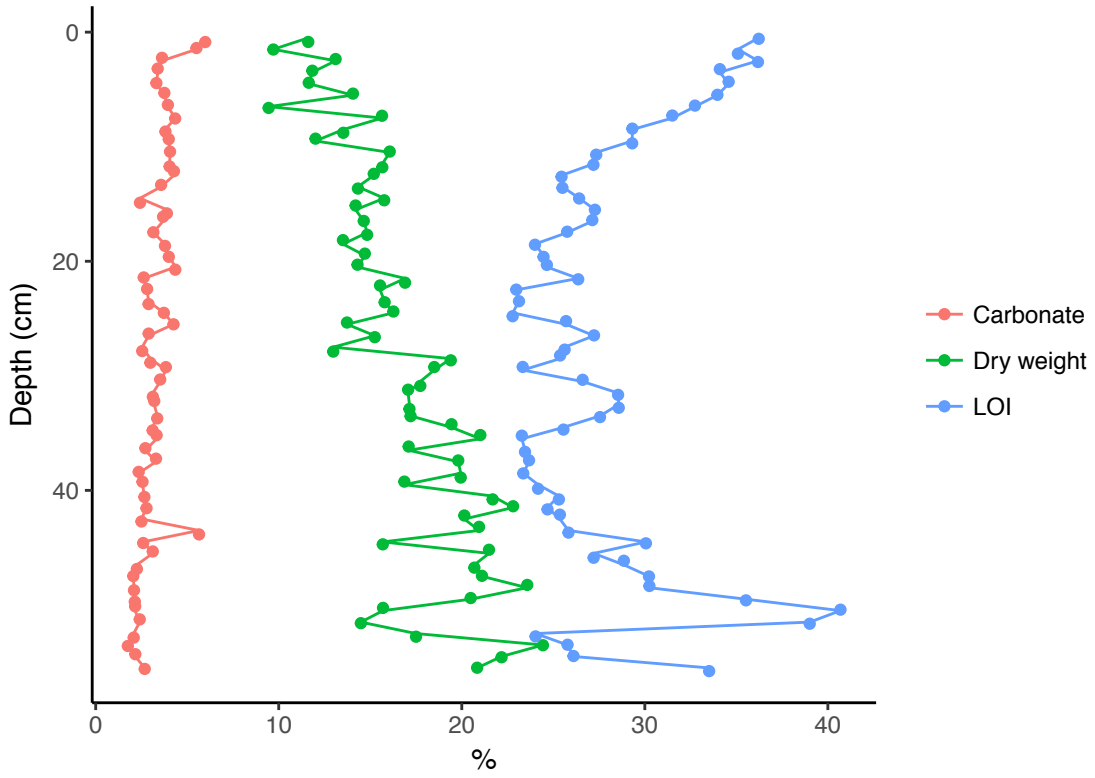

Figure 6.14 Changes in % dry weight, % loss on ignition and % carbonate in CRAIB.

Core Chronology

In CRAIB, equilibrium of total ^{210}Pb activity with supported ^{210}Pb activity is reached at ~36cm (Fig. 6.15a). Like ESTH9, the maximum value of unsupported ^{210}Pb activity is below the surface sediments, suggesting recent increases in sedimentation rates (Fig. 6.15b). Gradients of decline in unsupported ^{210}Pb activities increase with depth, suggesting higher rates of sedimentation towards the top of the core. The ^{137}Cs activity versus depth profile (Fig. 6.15c) shows one peak at 9.5cm and another at 15.5cm. These are likely derived from the 1986 Chernobyl fallout and the maximum fallout of atmospheric testing of nuclear weapons in 1963, respectively. The latter date is supported by ^{241}Am, which was detected between 11.5 and 17.5cm. The CRS model places 1986 at 12.5cm and 1963 at 18cm – deeper than suggested by ^{137}Cs and ^{241}Am. Chronologies and sedimentation rates were therefore corrected by referring sediments at 15.5cm as formed

in 1963. This puts 1986 at 9.5 – 11.5cm, in agreement with the ^{137}Cs record. Mean sedimentation rates were calculated at 0.025g cm^{-2} yr^{-1} before the 1930s, increasing to 0.067g cm^{-2} yr^{-1} after this date.

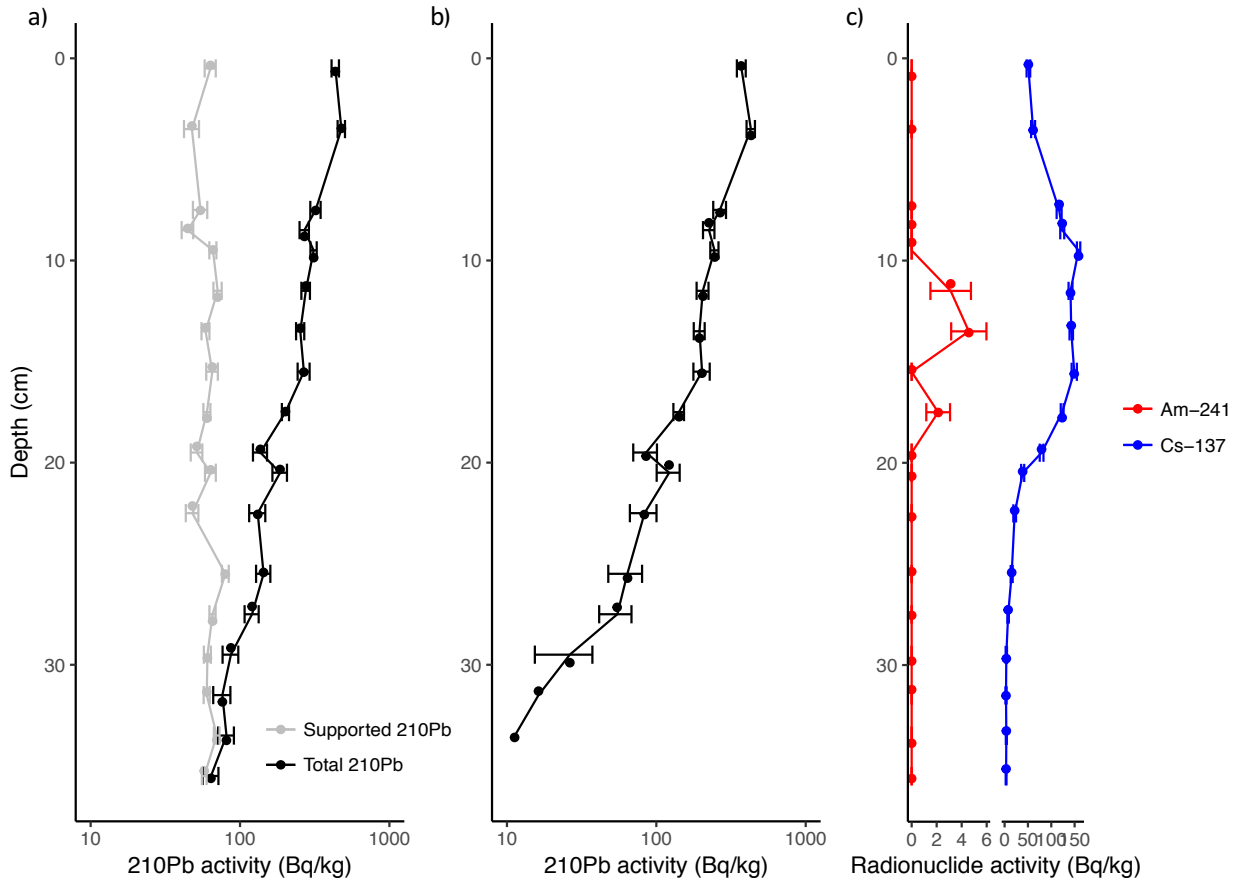

Figure 6.15 Fallout radionuclide concentrations in CRAIB: a) total ^{210}Pb; (b) unsupported ^{210}Pb; and c) ^{137}Cs and ^{241}Am.

Table 6.2 Core chronology of CRAIB.

Depth	Drymass	Chronology			Sedimentation Rate		
		Date	Age				
cm	g cm^{-2}	AD	yr	±	g cm^{-2} yr^{-1}	cm yr^{-1}	± %
0	0	2014	0				
0.5	0.0586	2013	1	2	0.0673	0.562	9.8
3.5	0.4192	2007	7	2	0.0477	0.399	10.6
7.5	0.8949	1998	16	2	0.0579	0.438	14.6
8.5	1.0808	1995	19	2	0.0622	0.355	14.8
9.5	1.2451	1992	22	3	0.0522	0.304	14.5
11.5	1.5959	1985	29	4	0.0506	0.253	18.3
13.5	2.0441	1975	39	5	0.039	0.189	23.1
15.5	2.4212	1963	51	5	0.042	0.21	33.2
17.5	2.842	1956	58	6	0.0639	0.286	40.5
19.5	3.3156	1949	65	6	0.0592	0.273	20.7
20.5	3.4929	1945	69	7	0.04	0.214	20.5
22.5	3.8754	1935	79	7	0.038	0.2	23.8
25.5	4.4424	1920	94	9	0.0257	0.151	30.7
27.5	4.7274	1908	106	11	0.0204	0.13	33.2
29.5	5.0689	1895	119	14	0.0267	0.142	51.6
31.5	5.4803	1879	135	16	0.0268	0.128	74.5
33.5	5.906	1862	152	17	0.0226	0.106	93.2

N. flexilis*: Macrofossils and historical records*

In CRAIB, *N. flexilis* seeds were found in relatively small numbers (max. 8 seeds per 100cm^3 wet sediment), in several but not all samples between 35cm and 4cm (pre-2007) (Fig. 6.16). *N. flexilis* leaf spines were found below 6cm. Historically, *N. flexilis* was recorded in the lake between 1967 and 2004, but has been absent in all surveys completed

since then (Murphy, 2007, unpublished; Benthic Solutions, 2007). Samples in CRAIB have therefore been split into two zones; low *N. flexilis* abundance (below 5cm) and *N. flexilis* absent (0 – 4cm). These zones are marked on Fig. 6.16.

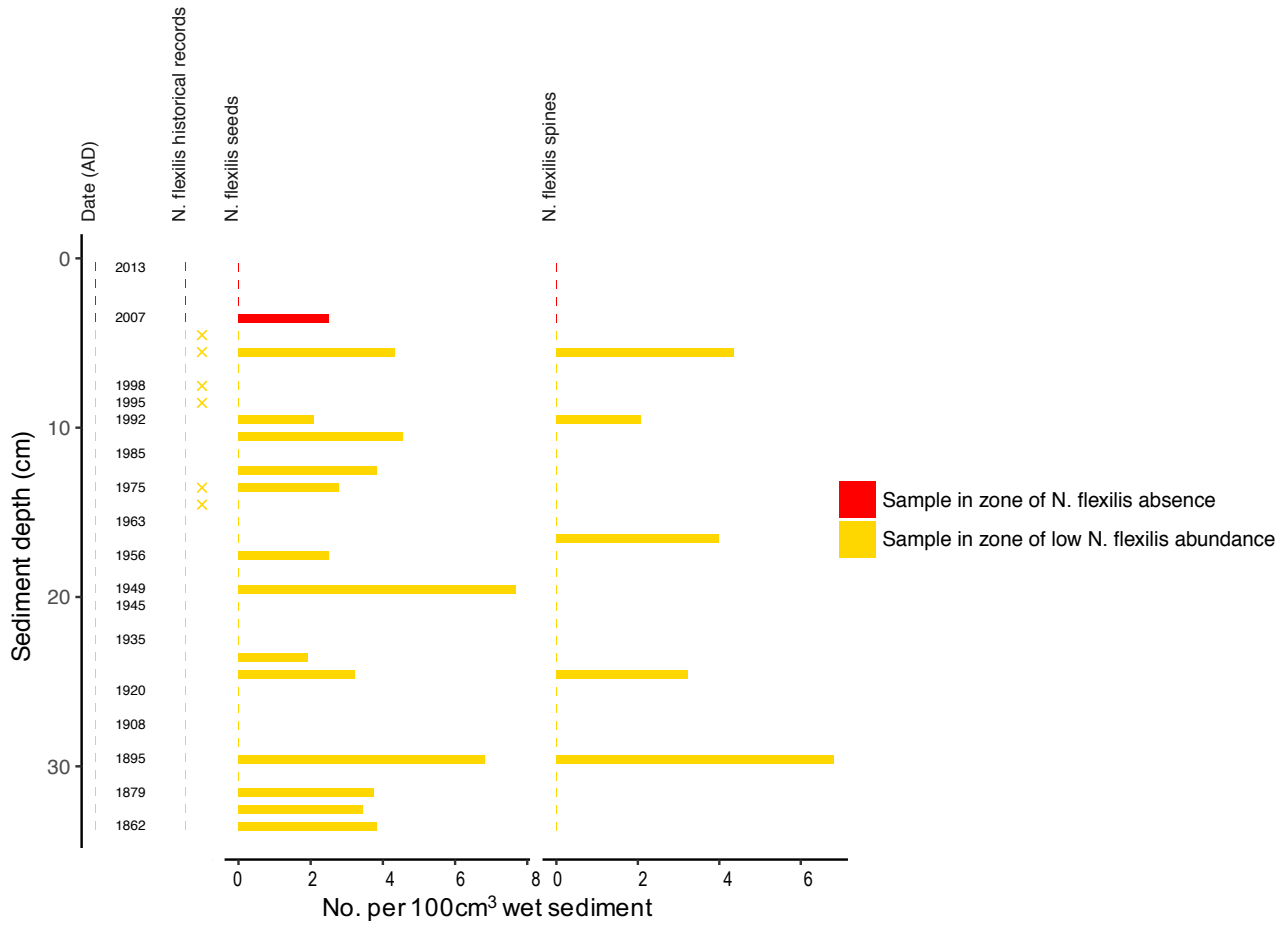

Figure 6.16 Changes in numbers of N. flexilis seeds and leaf spines in CRAIB, plotted alongside historical botanical records.

Macrofossils

There is no evidence for any major ecosystem-wide floristic change in the macrofossil remains in CRAIB. The first three axes of a PCA explain 51.84% of the variance in plant macrofossil data (Fig. 6.18), and highlight a few key species which explain the difference between samples representing low *N. flexilis* abundance and those representing *N. flexilis* absence. Fig. 6.17 shows that there was an increase in *Nymphaea* triclosclerids and a

decrease in *Potamogeton* remains above 5cm (post ~2007), and that *Pediastrum* appeared in the record at a similar time. *N. flexilis* remains were most closely associated with *Juncus bulbosus* and *Potamogeton gramineus* remains. Fig. 6.17 also shows a decline in *Juncus bulbosus, Nuphar lutea* and *Persecaria amphibia* and an increase in *Isoetes lacustris* and the large diatom *Surirella* sp. upwards of 20cm (~1945, and a small increase in *Nitella* sp. oospores upwards of 11.5cm (~1985), but these changes are not linked to the disappearance of *N. flexilis* in 2007.

There are some notable differences in the macrofossils associated with *N. flexilis* in CRAIB and ESTH9. The numbers of *Nitella* sp. and *Chara* sp. oospores in CRAIB are small throughout the core, and comparable to the numbers of *Characeae* remains found in the topmost section of ESTH9. In contrast, the number and diversity of *Potamogeton* remains was much higher in CRAIB. *Sphagnum* remains were abundant in CRAIB but absent from ESTH9.

Like the plant macrofossils, there are no major changes in faunal remains in CRAIB associated with *N. flexilis* loss. 55.51% of the variance in the data is explained within the first three axes of a PCA (Fig. 6.20). Fig. 6.19 shows that *Daphnia* ephippia and *Trichoptera* remains decreased in number above 26cm (~1920), *Chydoridae* carapaces increased above 12cm (~1985), and *Ceriodaphnia* ephippia and *Crystatella statoblasts* increased above 8cm (~1998), but these changes all occurred before the loss of *N. flexilis* in ~2007.

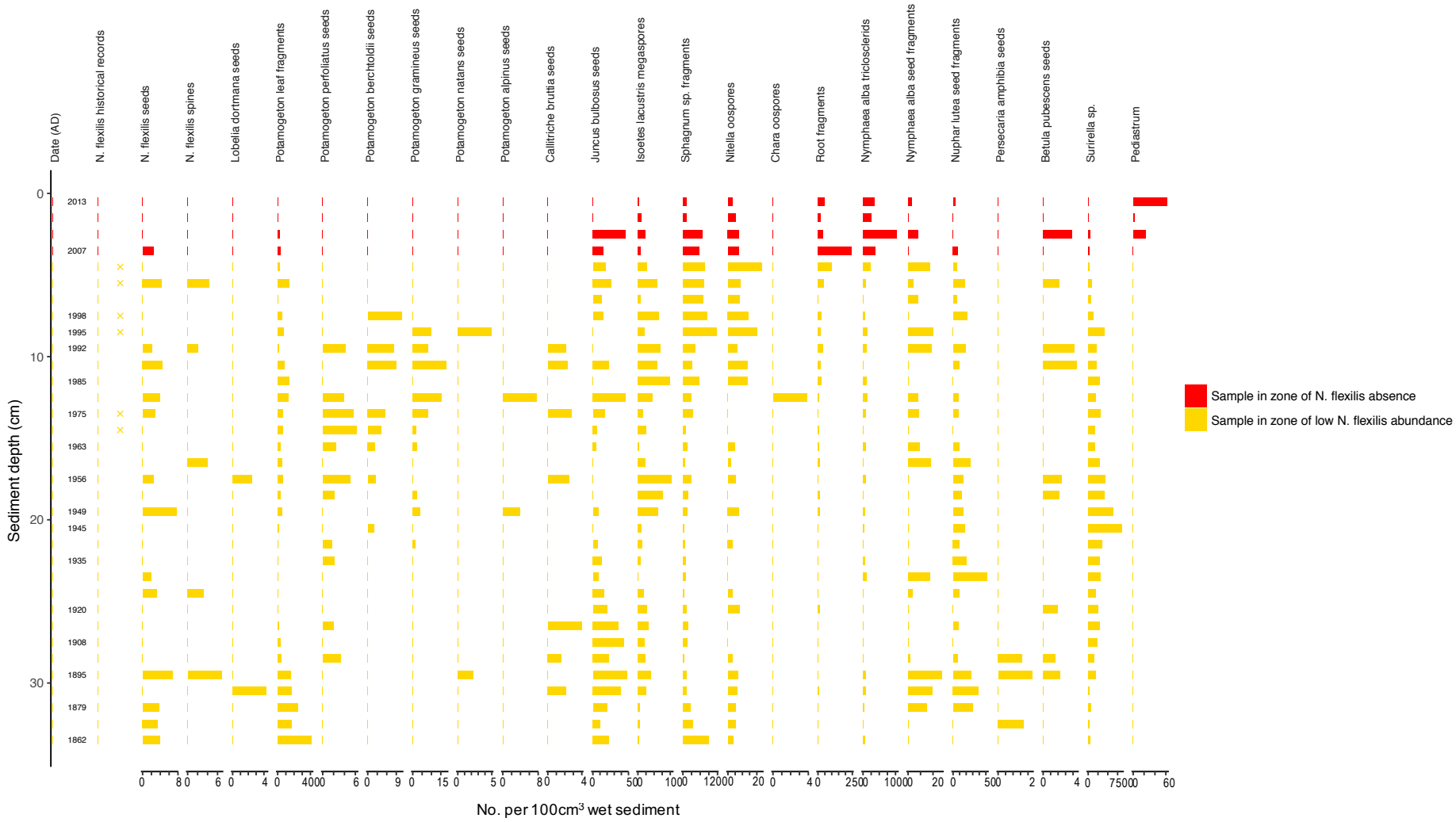

Figure 6.17 Changes in plant macrofossil numbers through time in CRAIB, colour-coded by N. flexilis *abundance and plotted alongside historical botanical records for* N. flexilis

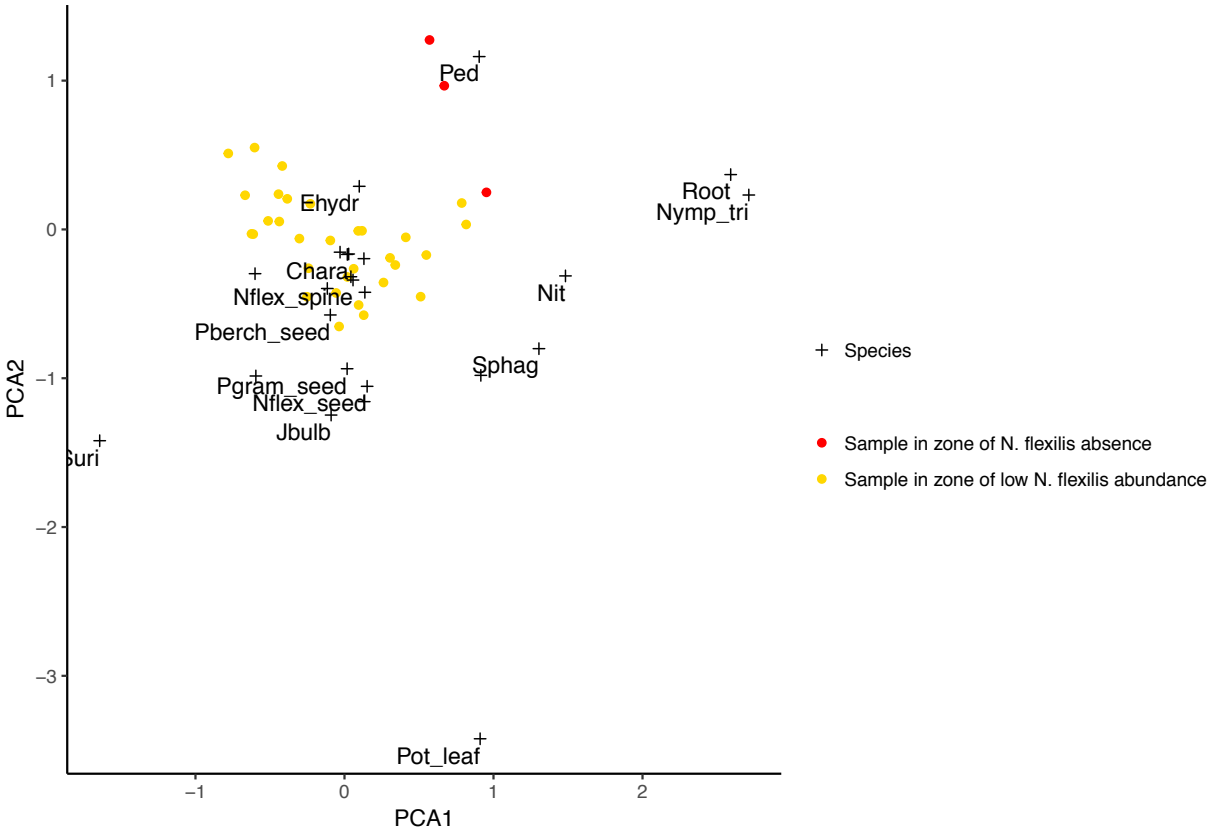

Figure 6.18 Principal Components Analysis of plant macrofossils in CRAIB, colour-coded by N. flexilis *abundance. Plotted axes explain 48% of variance. Species abbreviations are defined in Appendix 8.*

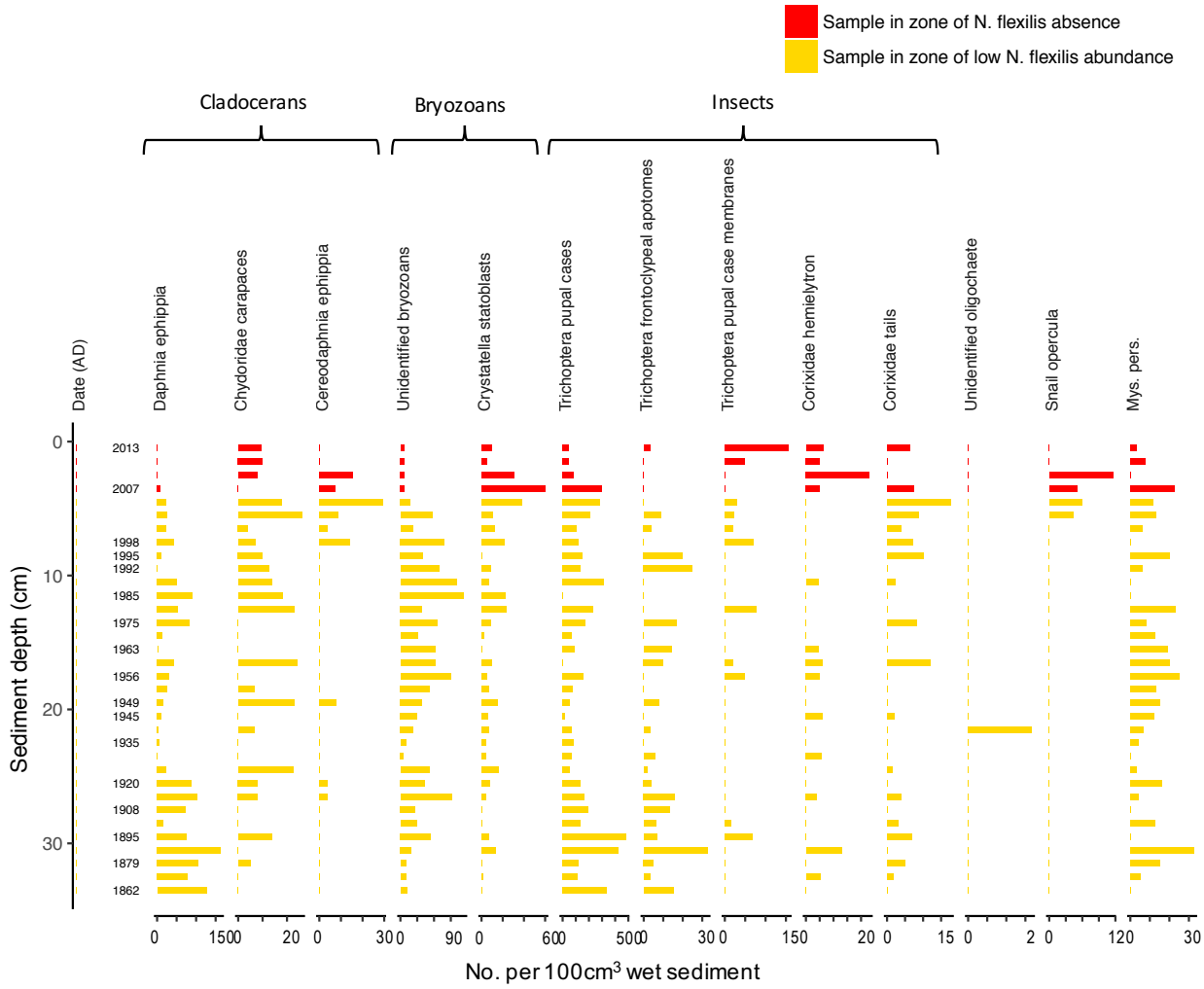

Figure 6.19 Changes in faunal macrofossil numbers through time in CRAIB, colour-coded by associated N. flexilis abundance.

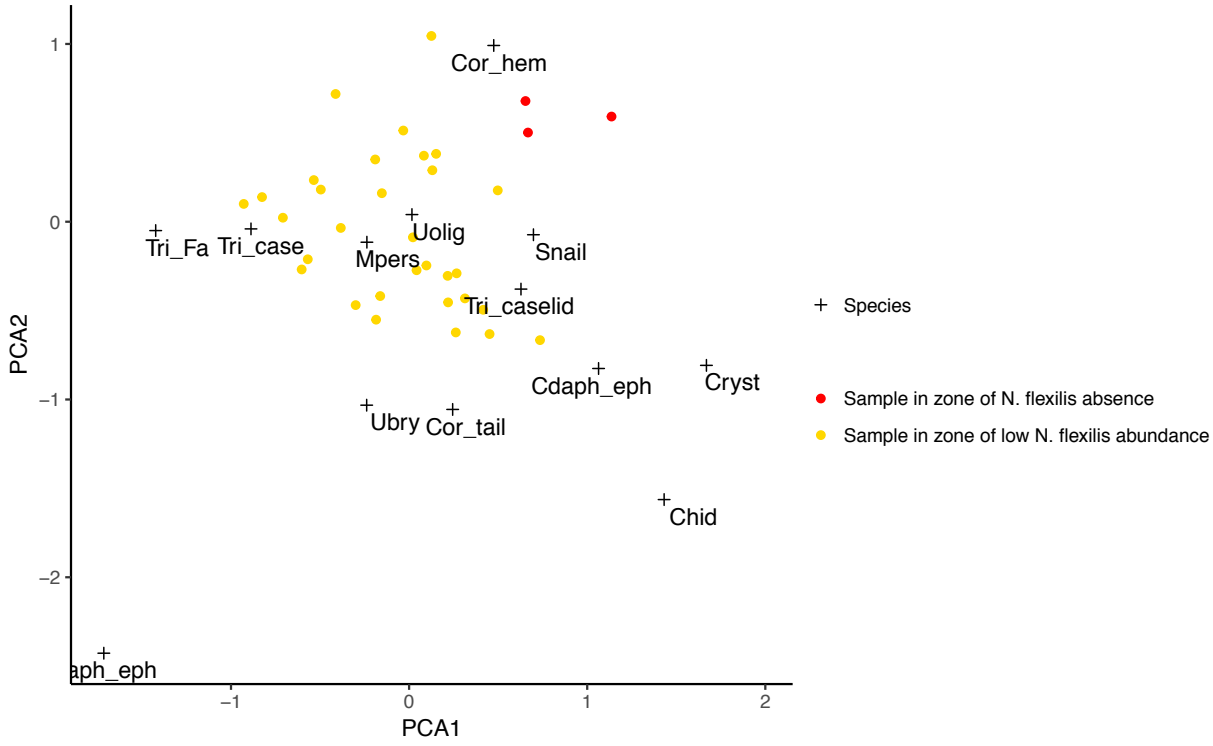

Figure 6.20 Principal Components Analysis of faunal macrofossils in CRAIB, colour-coded by associated N. flexilis *abundance. Plotted axes explain 48% of variance. Species abbreviations are defined in Appendix 8.*

Chitinous Cladocera remains

Fig. 6.21 shows changes in the Cladocera community represented in CRAIB, with a major ecological shift at ~12cm (~1985). Below this point, Chydorid species were dominant, particularly *Chydorus piger*, *Alonella nana* and *Alona affinis.* The presence of species with tubercles on their shells, such as *Chydorus piger* and *Alona guttata/rectangularis var tuberculosis* was noteable in this zone. Above ~12cm, the percentage abundance of *Bosmina* species increased, and *Chydorus piger* was replaced by an increase in *Chydorus sphericus*. There was also an increase in *Sida crystallina* and *Graptoloberis* sp. during this period. However, these changes were not associated with the loss of *N. flexilis,* and, in a PCA, samples associated with differing abundances of *N.*

flexilis were not grouped together (Fig. 6.22).

In comparison to ESTH9, the *Chydoridae* were both more abundant and more diverse in CRAIB, particularly the species *Alonella nana, Graptoloberis* sp, *Chydorus piger, Alonella exisa* and *Alonella exigua.* The exception to this is *Camptocercus rectirostris*, which was relatively abundant in the lower section of ESTH9 but rare throughout CRAIB.

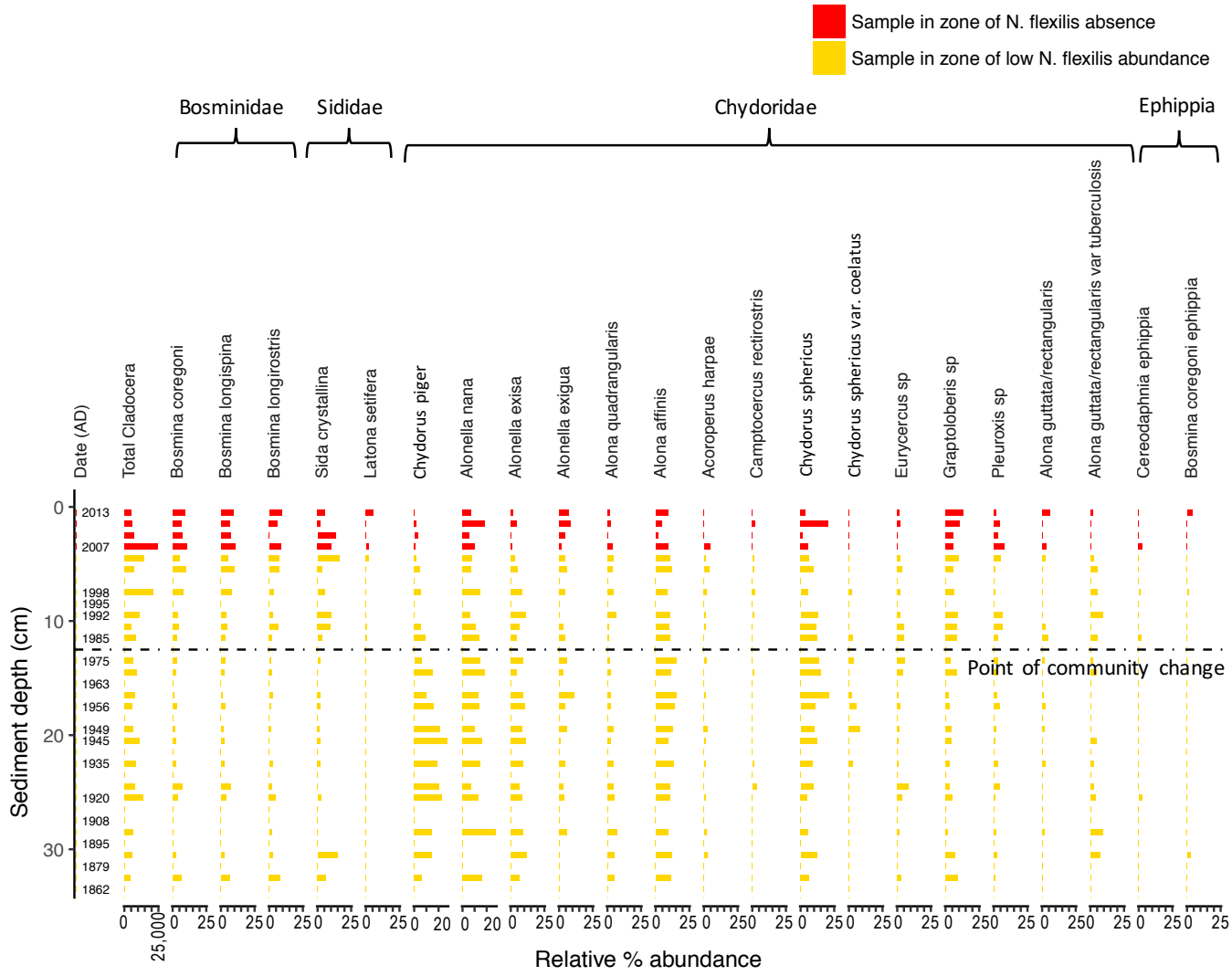

Figure 6.21 Changes in relative percentage abundance of Cladocera species through time in CRAIB, colour-coded by associated N. flexilis abundance. x-axis for total Cladocera shows absolute counts.

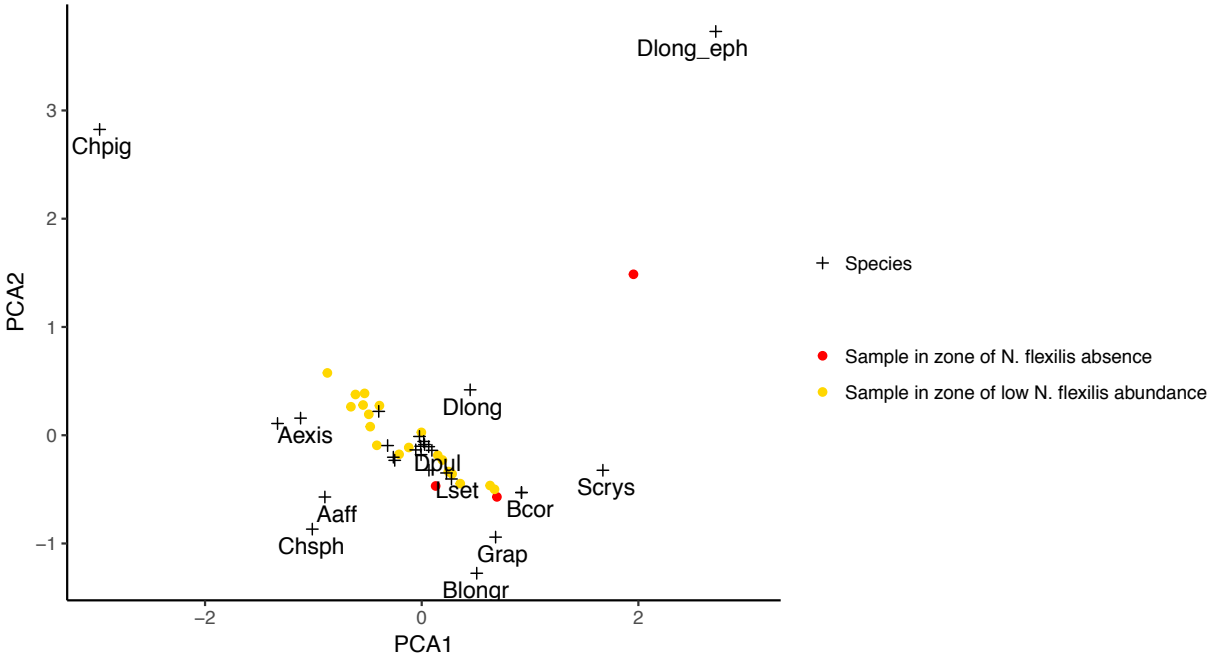

Figure 6.22 Principal Components Analysis of Cladocera species in CRAIB, colour-coded by associated N. flexilis *abundance. Plotted axes explain 52% of variance. Species abbreviations are defined in Appendix 8.*

6.2.5 Loch of Craiglush monitoring data

Fig. 6.23 shows water quality monitoring data from the inflow to Loch of Craiglush since 1987, compared to *N. flexilis* abundance. It should be noted that these data reflect the inflow, and not the water quality of the lake itself - it is therefore not possible to directly compare this data to the monitoring data from Esthwaite Water. The records from the inflow show that, in the decade preceding the loss of *N. flexilis,* concentrations of all measured nutrients increased, peaking in ~1997. Oxygen saturation dipped to ~90% at this time. Between 1996 and 1998, mean total phosphorus exceeded the annual mean WFD target of 15 ug l^{-1} for mesotrophic lakes (Interagency Freshwater Group, 2015).

However, since ~2000, nutrient inflows to Loch of Craiglush have recovered to their 1987 levels, and much of this recovery occurred before *N. flexilis* was last recorded in 2004. Although the alkalinity of the inflow has remained reasonably constant, pH has declined from ~7.75 to ~7.25 over the course of the monitoring program, with the lowest recorded pH levels coinciding with the loss of *N. flexilis*.

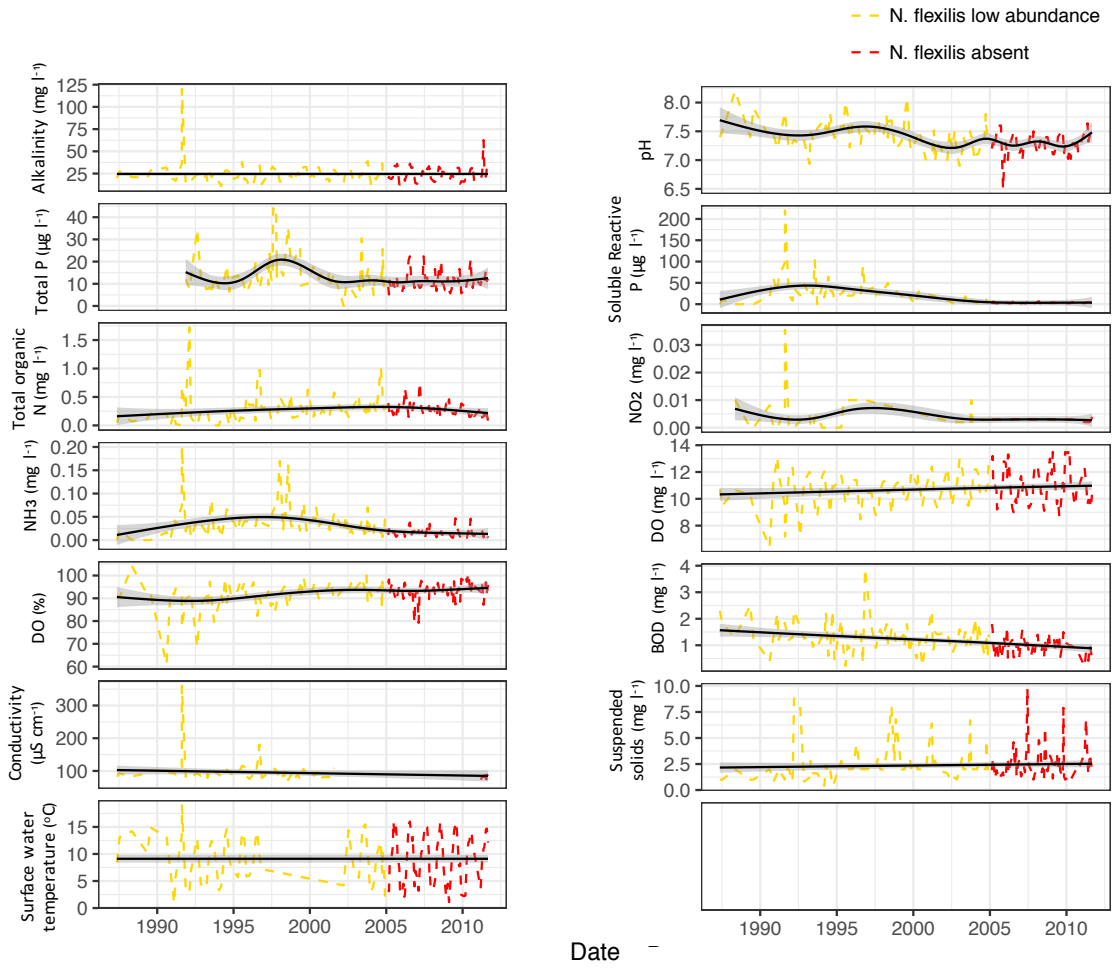

Figure 6.23 Water chemistry monitoring data from Loch of Craiglush between 1987 and 2014, with GAM smoothing (black line) to visualize trends. Records are colour-coded according to associated N. flexilis *abundance.*

Historical macrophyte surveys are available for Loch of Craiglush from 1986 - 2016 (Fig. 6.24). Alongside *N. flexilis, Potamogeton alpinus, Potamogeton berchtoldii* and *Subularia aquatica* have not been recorded since 2004. Since *N. flexilis* was lost from the

site, several other plant species have been recorded for the first time; *Chara virgata, Nuphar x spenneriana, Alnus glutinosa, Eleocharis acicularis, Phragmites australis* and *Sparganium angustifolium.* However, it is not known whether these species were present before 1986. The majority of species recorded at Loch of Craiglush were present both before and after the disappearance of *N. flexilis.*

Compared to the historical botanical records from Esthwaite Water, far fewer species have been recorded at Loch of Craiglush. Although many species were found at both sites, there were some species that formed a major component of the records at one site but weren't present at the other. At Esthwaite Water, *Potamogeton obtusifolius* and a diverse *Callitriche* flora were recorded frequently, whereas at Loch of Craiglush, *Juncus bulbosus* was a key species. A larger percentage of species recorded before the disappearance of *N. flexilis* persisted in more recent surveys at Loch of Craiglush than at Esthwaite Water, where there was a greater turnover in the plant community through time. At both sites, the loss of *N. flexilis* was associated with the appearance of *Phragmites australis* and *Sparganium angustifolium* in the records.

Figure 6.24 Historical macrophyte records from Esthwaite Water. Sources: Stewart, unpublished data (1986, 2007 and 2010), James and Barclay (1996), Howson et al (1997), and unpublished SCM data (2014).

6.3 Discussion

6.3.1 Esthwaite Water: The *N. flexilis* record

According to historical botanical records, *N. flexilis* was first recorded in 1914 during a shoreline survey (Pearsall, 1915). Because this was a shoreline survey and no attempt was made to collect plant material from water depths at which *N. flexilis* commonly grows, it is possible to speculate that *N. flexilis* must have been reasonably abundant at this time in order for plant fragments to collect on the shore. However, no indication of the recorded abundance of the plant is available from either this survey or from

subsequent surveys in which *N. flexilis* was recorded. The last documentary record of *N. flexilis* in Esthwaite Water dates from 1982 (National Biodiversity Network, 2014). The macrofossil record from ESTH9 broadly corresponds with the historical botanical records, but provides more information both on the status of *N. flexilis* pre-1914 and on the changing abundance of the plant over the last ~150 years. According to the numbers of seeds found in ESTH9, *N. flexilis* was relatively abundant until ~1915; this likely reflects the status of the plant as first encountered by Pearsall. From 1915, the number of seeds in ESTH9 suggest that the abundance of the plant steadily declined, with seeds only intermittently present between ~1938 and ~1987. The numbers of *N. flexilis* leaf spines also reflect this decline in abundance, but suggest that it occurred later, from ~1936. Although *N. flexilis* has not been recorded at Esthwaite Water since 1982, *N. flexilis* seeds reappear in ESTH9 in small numbers at ~2002. *N. flexilis* spines are not present in ESTH9 after ~1987. Attempts were made to re-introduce the species to the lake from seed in the late 2000s, but were unsuccessful (Walmisley, pers. comms). Given the error ranges (stated in table 6.1, page 155) on radiometric dates in this section of the core, it is possible that these seeds represent the reintroduction attempt.

6.3.2 Environmental changes at Esthwaite Water

The eutrophication of Esthwaite Water has been well documented using both long-term chemical monitoring and paleoecological reconstructions of both diatoms and algal pigments (Dong *et al.*, 2011; Dong *et al.*, 2012; Moorhouse, 2016; Maberly *et al.*, 2017), and is reflected in both the macrofossil and cladocera remains of ESTH9 and the diatom remains in ESTH1/ESTH7. Before ~1915, macrofossil remains suggest that the plant community was rich in submerged macrophytes including charophytes, *I. lacustris* and *N. flexilis,* reflecting a typical community of a relatively base-rich oligo-mesotrophic lake (Duigan *et al.*, 2006). In the cladocera record, *Sida crystallina* and *Camptocercus rectirostris* are more abundant below ~1936. In training sets based on Irish lakes, these species have been found to be strongly correlated with oligo-mesotrophic, base-rich lakes, and are particularly abundant at sites with high water clarity (Chen *et al.*, 2010). Pre-~1915, the diatom community was dominated by *Cyclotella comensis.* This species is

typically associated with oligotrophic conditions, with optimum SRP concentrations for the species generally set at <10 ug l^{-1} in diatom-environment training sets (e.g. Fritz *et al.*, 1993; Reavie and Smol, 2001).

Between ~1915 and ~1978, macrofossils indicated that submerged vegetation persisted, but that there was a community shift away from charophytes in favour of *Potamogeton* spp. The floating leaved *Nymphaeaceae* also increased. This suggests a shift towards vegetation more tolerant of eutrophic conditions. *Alona affinis* and *Chydorus sphericus,* which increase in abundance after ~1936, are epiphytic zooplankton associated mesotrophic, base-rich sites (Chen *et al.*, 2010), and suggest increased plant biomass during this period. From ~1915, *Cyclotella comensis* abundance declines, and *Fragilaria crotenensis* - a species typically seen in lakes during the early onset of eutrophication in hardwater lakes (e.g. Lotter, 1989; Lotter, 1998) - appears in the diatom record. Water chemistry monitoring, which began in 1945, confirms that Secchi depth decreased from ~4m to ~3m during this period, which could explain the loss of species that typically inhabit deeper waters, such as the macrophytes *Chara* spp. and *Nitella* spp. and the Cladoceran *Camptocercus rectirostris.* Nutrient concentrations rose slowly but steadily at this time, and were likely the cause of these ecological changes. These early nutrient inputs have been attributed to the increased draw of the area as a tourist destination and resultant increases in the human population, which lead to increased discharges both from domestic sewerage and from improved livestock pasture (Dong *et al.*, 2011; Moorhouse, 2016).

Post ~ 1978, *Nymphaeaceae* become more prominent in the macrofossil remains. This is often observed in sediment cores from sites undergoing eutrophication, where isoetids and elodeids are gradually replaced by canopy-forming, emergent and floating-leaved plants (e.g. Rasmussen and Anderson, 2005; Madgwick *et al.*, 2011). Historical macrophyte records show an increased occurrence of emergent species in surveys post 1995, including *Caltha palustris, Schoenoplectus lacustris, Typha latifola* and *Phragmites australis.* The increase in chitinous *Daphnia* remains after ~1968 also implies increased primary production. Typically, in shallow lakes, *Daphnia* populations tend to

increase as phytoplankton – their food source – increases (Lau and Lane, 2002; Davidson *et al.*, 2010). Dong *et al.* (2011) used the changing ratio of benthic to planktonic diatoms in ESTH7 as evidence for nutrient enrichment from ~1976 onwards, particularly the dominance of *Stephanodiscus hantzschii* and *Fragilaria crotenensis* after this date. *Tabellaria flocculosa* – a species associated with the early stages of nutrient enrichment (Alefs and Müller, 1999) – also declined during this period, suggesting an increase in the intensity of nutrient enrichment. In sediment cores taken from Esthwaite Water by Moorhouse (2016), algal pigments associated with filamentous cyanobacteria were highest after ~1960, reflecting summer algal blooms likely linked to eutrophication. In 1973, the Hawskhead sewage treatment works were opened in the Esthwaite Water catchment, and fish cages were introduced in 1981 (Dong *et al.*, 2012). The increased nutrient input from these point sources is reflected in the chemical monitoring record, which shows faster rates of increase in all forms of phosphorus and nitrogen, a sharp decline in Secchi depth, and a decline in surface oxygen saturation after 1980. The chemical and ecological changes that occurred post ~1973 have been widely attributed to these anthropogenic sources of pollution (Bennion *et al.*, 2000; Dong *et al.*, 2011; Dong *et al.*, 2012). This includes the increased alkalinity evident in the chemical monitoring record, which has been attributed to changes in organic carbon speciation (specifically an increase in the ratio of HCO_{3-} to CO_2) as photosynthesis rates increased as a result of eutrophication (Maberly, 1996).

Tertiary treatment was added to the Hawkshead works in 1986, and the fish cages were removed in 2009 (Dong *et al.*, 2012). Water chemistry monitoring suggests that nutrient concentrations began to decrease in the lake post ~2000, and that Secchi depth has recovered to ~3m from a previous low of ~2m. In the top 3cm of ESTH9, a small increase in the numbers of *Chara* and *Nitella* oospores, a decrease in *Chydorus sphericus,* and increases in *Bosmina longispina* and *Acroperus harpae* may be the first hints of ecological recovery. However, releases of phosphorus stored in the sediment as well as diffuse agricultural pollution mean that the onset of this recovery has been slow, and continued increases in alkalinity reflect the fact that plant biomass in the lake remains high. Whilst nutrient inputs remain the primary concern, climate change also has the

potential to influence the ecology of Esthwaite Water (Dong *et al.*, 2012), and surface water temperatures continue to rise steadily. Invasive species are also an ongoing concern, with both *Elodea canadensis* and *Elodea nutallii* present. *Elodea canadensis* was first recorded by Pearsall in 1914, but the abundance of the plant and the nature of it's colonization of the site is not known and the species is not represented in macrofossil remains. *Elodea nuttallii* was first recorded in 2008, and was described as "the most dominant and widespread species" in 2011 (Maberly, 2011: p101).

6.3.3 Causes of *N. flexilis* decline at Esthwaite Water

Before ~1915, *N. flexilis* was relatively abundant at Esthwaite Water, and, according to the macrofossil record and the results of Pearsall's 1914 botanical survey, grew alongside a plant community typical of a mildly alkaline lake, characterised by the presence of *Chara* sp., *Nitella* sp., *Potamogeton gramineus, Potamogeton perfoliatus, Isoetes lacustris* and *Lobelia dortmana.* The timing of the initial decline of *N. flexilis* from peak abundance at Esthwaite Water corresponds with the ecological changes that occurred from around ~1915, when nutrient levels began to increase and water clarity to decline. At this time, macrofossils indicate an increase in *Potamogeton* spp. Early water chemistry monitoring indicates alkalinities between 15 and 20 mg l-1 and a pH ~7.5. In chapter 4, *N. flexilis* was shown to live in relatively high abundances in these conditions, which most closely resemble those of division 3 sites. However, division 3 sites with the highest abundances of *N. flexilis* were associated with high water clarity. In such sites, *N. flexilis* occupies deep water, where it has a competitive advantage over macrophytes that are able to use bicarbonate for photosynthesis. These bicarbonate users are likely limited by phosphorus availability. From ~1915, there is evidence not only for increases in nutrient availability at Esthwaite Water (e.g. increasing abundances of *Potamogeton* spp., macrophyte-associated Cladocerans and *Fragilaria crotenensis*, and declining abundances of *Cyclotella comensis*), but also for decreases in deep-water species that favour high water clarity (e.g. *Chara* spp., *Nitella* spp., *Camptocercus rectirostris*). It is likely that, at this time, the deeper waters favored by *N. flexilis* were becoming darker, whilst available CO_2 was being rapidly consumed in shallower waters by the growth of

other plant species, spurred by increases in nutrient availability. Thus less *N. flexilis* habitat was available, and the abundance of the species declined. It is possible that *E. canadensis* was a significant competitor of *N. flexilis* at this time, although there is a lack of evidence available to support this.

Although *N. flexilis* was significantly affected by the early stages of eutrophication at Esthwaite Water, it persisted until the opening of the Hawkshead treatment works and the introduction of the fish farm in the 1980s. The nutrient inputs from these sources had a significant effect on the ecology of Esthwaite Water evident in the macrofossil, cladocera and diatom records (Dong *et al.*, 2011; 2010), the algal pigments (Moorhouse, 2016), water chemistry monitoring (Maberly *et al.*, 2011) and historical botanical records (Bennion *et al.*, 2000). The disappearance of *N. flexilis* corresponds with the timing of these changes. Water chemistry monitoring shows a significant reduction in Secchi depth, as well as an increase in alkalinity. Monitoring records of pH, temperature and alkalinity from 1993 confirm that HCO_3^- was the dominant form of organic carbon in the lake during the *N. flexilis* growing season at this time (Maberly, 1996). At this point, it is likely that *N. flexilis* was unable to survive the combination of low availability of carbon dioxide for photosynthesis in the majority of the photic zone and low availability of light in the deeper waters away from bicarbonate-utilizing species.

Recent attempts to reintroduce *N. flexilis* to Esthwaite Water have been based on the fact that the chemistry and ecology of Esthwaite Water are returning to pre-~1980s levels, when *N. flexilis* disappeared. This study reveals that *N. flexilis* was under threat at the site since ~1915. Although light penetration at Esthwaite Water has begun to recover, alkalinity remains high and has yet to begin to decrease. Furthermore, the prevalence of *E. nuttallii* in recent years provides an additional source of competition to new *N. flexilis* plants. This may explain why reintroduction attempts have failed thus far; whilst the ratio of bicarbonate to dissolved CO_2 is still high, *N. flexilis* remains at a competitive disadvantage at Esthwaite Water.

6.3.4 Loch of Craiglush: The *N. flexilis* record

The first historical botanical record of *N. flexilis* from Loch of Craiglush dates back to 1967 (National Biodiversity Network, 2014), however no indication of the abundance of *N. flexilis* at the site is given until 1994, when the plant was found growing in just one small location within the loch, albeit fairly densely within that patch (James and Barclay, 1996). Observations of similar local abundance but lake-wide rarity were also made in 1995, 1996 and 1997 (James and Barclay, 1996; Howson, Mercer and Hogg, 1997), but in 2004 only a few plants were found (Murphy, 2007, unpublished). *N. flexilis* seeds and spines were found in small numbers throughout CRAIB, reflecting the fact that the species has likely been present in low abundance since pre-1850. There was no evidence for any significant changes in the abundance of *N. flexilis* through the core, although there are short periods during which seeds were not present. It is not clear whether these periods of absence reflect an absence of *N. flexilis* from the loch, or are a result of a patchy distribution of the plant preventing the dispersal of seeds to the coring site. Although botanical records indicate that *N. flexilis* was last recorded in Loch of Craiglush in 2004, seeds were found in sediments towards the top of CRAIB dating from ~2007. This is likely a "false-positive", similar to those found at Loch of Butterstone and Loch Flemington (see chapter 5). In line with the historical records, which were derived from extensive snorkel and SCUBA surveys and therefore considered reliable, *N, flexilis* was considered "absent" from 2004 onwards during analysis and interpretation of CRAIB.

6.3.5 Environmental changes at Loch of Craiglush

The paleoecological record provides little evidence for any major ecosystem-wide ecological changes in Loch of Craiglush between 1850 and the present, both in terms of the macrofossils in CRAIB and the diatoms analysed by Finney (1998). The macrofossil remains found in CRAIB reflect a relatively acid-tolerant flora, comprised of *Potamogeton* spp., *Juncus bulbosus, Sphagnum* spp., and *Nuphar lutea. Nitella* oospores are present in very low numbers throughout the core, but *Chara* spp., which prefer base-rich conditions (Duigan *et al.*, 2006), are predominantly absent. This is confirmed by

botanical records, which indicate the presence of a large isoetid community including *Isoetes echinospera, Isoetes lacustris, Littorella uniflora* and *Lobelia dortmana.* The Cladocera remains are dominated by macrophyte-associated taxa commonly found in acid/circumneutral lakes with low nutrient concentrations, such as *Graptoloberis* spp., *Chydorus piger* and *Alona guttata/rectangularis* (Chen *et al.*, 2010; Davidson *et al.*, 2010). Diatom analysis of a pelagic core from Loch of Craiglush indicates a community dominated by circumneutral taxa including *Achnanthes minutissima*, *Brachysira zelensis* and *Cyclotella radiosa* (Finney, 1998). Contemporary water chemistry monitoring of the inflow suggests that water entering Loch of Craiglush has a pH of ~7, and that nutrient concentrations entering the lake from the inflow are low (<25 ug l-1 total P and <30 ug l-1 total organic N). The underlying geology of Loch of Craiglush, which is comprised of mica-schists and quartzites, suggests that the lake is likely to retain the circumneutral pH of its inflow, whilst the low nutrient inflows imply that alkalinity changes resulting from increased primary production are unlikely to occur.

Some changes were noted in the Cladocera record in CRAIB, where there is a shift from *chydoridae* dominance to dominance of *Bosmina* spp. after ~1985. This is accompanied by a simultaneous increase in the numbers of chydorid carapaces (ephippia) in the macrofossil record. *Chydoridae* are known to exhibit increased sexual reproduction as a response to environmental stress (Nevalainen *et al.*, 2011b), and it is likely that there is some kind of environmental pressure affecting the *chydoridae* at the coring site at this time. *Bosminidae* are predominantly pelagic species, whilst *chydoridae* are associated with the littoral benthos (Frey, 1988). It is therefore common to infer changes in lake water level from changes in the relative abundance of *Bosminidae* and *chydoridae* (Hofmann, 1998). However, there is no historical evidence for lake water level change at Loch of Craiglush during the 19th century; current water levels and bathymetry closely resemble those mapped between 1897 and 1909 (Murray and Pullar, 1910). The macrofossil record does not indicate a loss of plant species consistent with the type of increase in turbidity that would cause a change in the depth boundary between the littoral and pelagic zones, but rather indicates a slight increase in the abundance of species that favour high water clarity (e.g. *Nitella* spp. And *Isoetes lacustris*) from ~1985. The timing

of the change coincides with the introduction of fish cages into Loch of Butterstone, which is adjacent to and hydrologically linked with Loch of Craiglush, in 1981. High densities of zooplanktivourous fish are typically associated with high relative abundances of *Bosminidae* in sediments, as predation pressure by *Daphnia* spp. is reduced (Jeppesen *et al.*, 1996; Davidson *et al.*, 2010). It is there possible that the increased abundance of *Bosminidae* in CRAIB reflects upstream migration of fish from Loch of Butterstone into Loch of Craiglush. However, this does not explain the simultaneous increase in macrophyte-associated cladocerans, such as *Graptoloberis* sp.. In order to fully understand this change, simultaneous changes in the sediment record of Loch of Butterstone should be explored. Since this change does not coincide with changes in *N. flexilis* abundance, such investigations are beyond the scope of this study.

6.3.6 Causes o*f N. flexilis* decline at Loch of Craiglush

Both the macrofossil record and monitoring records suggest that the abundance of *N. flexilis* at Loch of Craiglush has always been relatively low, and that the species grew alongside a plant community typical of a circumneutral/midly acidic lake, characterised by *Juncus bulbosus, Potamogeton gramineus* and *Sphagnum* spp. In chapter 4, these conditions reflected those found in the more acidic sites of division 2 (i.e. subgroup c), where *N. flexilis* was found in <20% of sample points. There are periods of time during which *N. flexilis* was notably absent from the macrofossil record (e.g. ~1879 - ~1920), and, given the low tolerance of *N. flexilis* to acidic conditions, it is possible that these absences are linked to short-term fluctuations in pH, however there is no evidence to support this. Since the disappearance of *N. flexilis* in ~2004, the inflow to Loch of Craiglush fell below pH 7 only for short periods, with a minimum recorded pH of 6.5 on 10th November 2005. Although this is towards the lower end of the pH tolerance range of *N. flexilis,* the plant has been observed to reproduce at pH 6.5 in the laboratory (Titus and Hoover, 1991). It is therefore unlikely that changes in pH are responsible for the contemporary loss of *N. flexilis* at Loch of Craiglush, despite the relatively acidic nature of the site. Similarly, there are no indications in either the monitoring record or the sediment record for significant nutrient inputs or for ecological responses to

eutrophication that may have affected water clarity or CO_2 availability to a large enough degree to remove *N. flexilis. Elodea canadensis* has been present at Loch of Craiglush since the 1950s (National Biodiversity Network, 2014) and, in 2007, was reported to be "extensive" (Benthic Solutions, 2007). However, the results presented in chapter 4 suggests that *Elodea* spp. and *N. flexilis* can co-exist in the absence of nutrient enrichment. This, alongside the fact that the invasion history of *Elodea canadensis* in Loch of Craiglush is unknown and impossible to infer from the macrofossil record, means that it cannot be determined whether *Elodea canadensis* invasion was responsible for *N. flexilis* loss at this site.

6.3.7 *N. flexilis* and environmental change at Loch of Butterstone and Loch of Lowes

Loch of Craiglush is part of a chain of hydrologically linked lochs, two of which (Loch of Lowes and Loch of Butterstone) are located in the immediate vicinity of Loch of Craiglush. Because of the close hydraulic links between the three sites and the high potential for species and environmental contaminants to disperse between the sites, examining a single loch in isolation of the other two risks neglecting important variables that influence the ecology of the lochs. Indeed, the extinction of *N. flexilis* in Loch of Craiglush occurred during the same year as Loch of Butterstone, and only three years before the plant was last recorded in Loch of Lowes. Whilst the littoral sediment core taken from Loch of Lowes for this study was not suitable for paleoecological analysis because of slow sediment accumulation rates, a pelagic core (LOWE2) was taken from this site and analysed for diatoms by Bennion *et al.* (2004). Both a littoral (BUTT5) and a pelagic core (BUTT4) were taken from Loch of Butterstone by Bennion *et al.* (2010) and analysed for macrofossils and diatoms respectively. In combination with the three short Glew cores (BUTT9, BUTT10 and BUTT11) from Loch of Butterstone presented in chapter 5, in which changes in *N. flexilis* seed numbers were counted, these cores provide some insight into the ecological changes that have occurred at these sites over the past ~150 years.

The maximum numbers of *N. flexilis* seeds per 100 mg wet sediment found in the cores

from Loch of Butterstone ranged from 11 (15cm sediment depth) in BUTT9 to 30 (40cm sediment depth) in BUTT4. According to the results presented in chapter 5, this implies that *N. flexilis* was formerly abundant at the site. This is reflected in historical botanical records, where dense stands of *N. flexilis* have been sighted in different locations in the eastern half of the lake between 1982 and 2004 (N. Stewart, pers. comm.; James and Barclay, 1996; Howson *et al.*, 1997; Murphy, 2007, unpublished data). Although macrofossil data are not available from Loch of Lowes, multiple historical botanical surveys dating from 1987 to the last sighting of *N. flexilis in* 2007 all report high abundances of the plant within the 1.5-2m isobath around the entire circumference of the site (James and Barclay, 1996; Howson *et al.*, 1997; Benthic Solutions, 2007). In all four cores from Loch of Butterstone, *N. flexilis* remains disappear suddenly, but at different depths in each core. Neither BUTT4, BUTT9, BUTT10 nor BUTT11 were successfully radiometrically dated, so whether the timing of this disappearance aligns with the last historical record of *N. flexilis* at the site in 2004 is unknown.

Ecosystem-wide changes are evident in the littoral and pelagic cores from Loch of Butterstone, and in the pelagic core from Loch of Lowes. Preservation of macrofossils in BUTT5 was poor, however the remains that were found below ~25cm represent a community dominated by *Nitella, Chara* and *N. flexilis,* typical of a mildly alkaline, mesotrophic lake (Bennion *et al.*, 2010). Diatom analysis of BUTT4 also suggests a reference community typical of circumneutral/mildly alkaline conditions, with *Achnanthes minutissima* the dominant species before ~1940 (Bennion *et al.*, 2010). The same was true of the pre ~1936 diatom community of a core taken from Loch of Lowes (LOWE2) (Bennion *et al.*, 2004). At both Loch of Butterstone and Loch of Lowes, the relative abundances of *Achnanthes minutissima* declined and *Fragilaria crotenensis* and *Aulacoseira subarctica* increased from the 1940s onwards (Bennion *et al.*, 2004; Bennion *et al.*, 2010). This increase in the number of planktonic species is indicative of a shift towards more mesotrophic conditions, and echoes the initial stages of eutrophication observed at Esthwaite Water. At Loch of Butterstone, the shift grew more pronounced in ~1980, when the relative abundance of *Achnanthes minutissima* suffered a rapid decline (Bennion *et al.*, 2010). In BUTT5, an increase in *Isoetes lacustris* megaspores, a decrease

in *Nitella* oospores and the appearance of *Potamogeton crispus* remains above ~30cm is indicative of increased vascular plant growth likely following nutrient enrichment (Bennion *et al.*, 2010). However, since BUTT5 was not dated, it was not possible to determine the timing of this ecological shift. Ecological changes at Loch of Butterstone and Loch of Lowes have been attributed to eutrophication from diffuse agricultural sources, as well as nutrient inputs and increased fish predation following the introduction of fish cages in Loch of Butterstone in 1980 (Finney *et al.*, 1998; Bennion *et al.*, 2010). These nutrient inputs are reflected in the water quality monitoring records from the outflows of Loch of Butterstone and Loch of Lowes. These show increasing nutrient concentrations between the start of monitoring in 1987 and the late 1990s, when levels of soluble reactive phosphorus, total phosphorus, ammonia, nitrates and nitrites peaked. At both outlets, peak nutrient concentrations were associated with increased alkalinity, possibly indicating changes in the speciation of organic carbon in the lakes as a result of increased photosynthetic rates.

The timing of the vegetative changes in BUTT5 corresponds with the disappearance of *N. flexilis* seeds from the core. However, because this core is not dated, it is not possible to infer whether this coincides with the last historical record of *N. flexilis* in ~2004. The diatom records from the dated pelagic cores suggest that nutrient enrichment and associated ecological changes have been happening at Loch of Lowes and Loch of Butterstone since the ~1940s, and, in the case of Loch of Butterstone, with some intensity since the introduction of fish cages in 1980 (Finney, 1998; Bennion *et al.*, 2004; Bennion *et al.*, 2010). Summer algal blooms have been reported in the Dunkeld-Blairgowrie lochs regularly over the last ten years - in 2010, Secchi depths in Loch of Butterstone were 0.75 – 1m (SEPA, unpublished data). However, the elevated nutrient levels and consequent reductions in light penetration do not appear to have been prolonged enough to have caused drastic ecological change. *Potamogeton crispus* remains, for example, are present in the upper portions of BUTT5, and *Achnanthes minutissima* - a species associated with low nutrient concentrations (Reavie and Smol, 2001) - retains a relatively high relative abundance at the top of BUTT4 and LOWE2 despite slight declines from reference conditions (Bennion *et al.*, 2004; Bennion *et al.*, 2010). At Esthwaite Water, *N. flexilis*

declined in abundance during the early stages of eutrophication but persisted at low abundances. It is possible that the relatively small ecological changes observed at Loch of Lowes and Loch of Butterstone as a result of eutrophication since ~1940 placed similar stress on *N. flexilis,* particularly in the light of the observed increases in measured alkalinity in the outflows. In 2007, *N. flexilis* was only growing in Loch of Lowes in water depths below 1.5 m (Scottish Natural Heritage, 2007); this may reflect the fact that carbon dioxide for photosynthesis was only available in deeper waters, away from other bicarbonate using plants. However, this pressure is not reflected by any significant declines in the numbers of *N. flexilis* seeds in BUTT5, BUTT9, BUTT10 or BUTT11, where seeds disappeared relatively quickly without a protracted period of reduced abundance. Furthermore, inputs into the Dunkeld-Blairgowrie lochs have been reducing since the mid 2000s following the implementation of catchment management strategies (Scottish Natural Heritage, 2004), and these measures have resulted in reductions in nutrient concentrations in the monitoring record that pre-date the loss of *N. flexilis* from the lochs. It therefore seems unlikely that nutrient enrichment is solely responsible for the disappearance of the species at Loch of Butterstone or Loch of Lowes.

The invasive species *Elodea canadensis* and *Elodea nutalii* were first recorded in the Dunkeld-Blairgowrie Lochs in the 1950s (National Biodiversity Network, 2014). However, their populations have apparently boomed since the mid-late 2000s. In 2007, Loch of Butterstone was described as having experienced "rampant growth" of *Elodea canadensis* (SNH, 2007). Both *Elodea canadensis* and *Elodea nuttalii* were present in Loch of Lowes in 2007, albeit within a narrow zone and not overlapping with *N. flexilis* populations (SNH, 2007). However, during the SCM in 2012, *Elodea* sp were present in 43% of sample points at Loch of Butterstone and 19% of sample points at Loch of Lowes (SNH, 2012a; SNH, 2012b). Unlike *N. flexilis*, *Elodea* spp. can switch from using CO_2 to using bicarbonate for photosynthesis, allowing it to maintain its growth rates under conditions of low CO_2 typically found where primary productivity is high (Jones *et al.*, 1993). *Elodea* plants are typically taller than *N. flexilis* plants, and it is not uncommon for *Elodea* spp. to shade out other macrophytes (Simpson, 1984). Furthermore, *Elodea* plants can suppress the growth of surrounding algae and cyanobacteria by secreting allelopathic

chemicals (Erhard and Gross, 2006). In conditions of elevated nutrient availability, the competitive advantage that *Elodea* spp. have over *N. flexilis* is therefore enhanced. In Loch of Butterstone and Loch of Lowes, the timing of the arrival of *Elodea* spp. in ~1950 coincided with changes in the diatom record indicative of mild eutrophication (evident from ~1940), and the expansion of *Elodea* spp. in the 2000s coincided with peak nutrient concentrations. Thus, *Elodea* spp. were likely already competitive within the *N. flexilis* habitat as nutrient concentrations began to decline, and, in tandem with eutrophication, may have contributed to the eventual loss of *N. flexilis* in the lochs. However, it is not possible to infer this from the paleoecological record, and further study is required to elucidate the relative effects of *Elodea* spp. and eutrophication on *N. flexilis* at these sites.

Whilst eutrophication has caused changes in the diatom flora of Loch of Lowes and Loch of Butterstone, there is no evidence for significant ecological changes as a result of nutrient enrichment at Loch of Craiglush. Finney (1998) suggested that this is because the catchment of Loch of Craiglush, unlike the other two lochs, does not support arable farming and the lake has therefore not been subject to the same nutrient loading. Furthermore, there are notable differences in the flora represented in the macrofossils from the two sites. Loch of Craiglush appears to have a relatively acid-tolerant flora, whilst the reference conditions of Loch of Butterstone reflect a flora dominated by Charophytes, which are typically associated with mildly alkaline lakes (Duigan *et al.*, 2006). Following analysis of the subfossil diatoms from cores taken from Loch of Craiglush, Loch of Lowes, Loch of Butterstone, Loch of Clunie and Loch of Marlee, Finney (1998) also observed that the flora of Loch of Craiglush was different from that of the other lochs, and representative of more acid-circumneutral conditions. This likely explains the lower abundance of *N. flexilis,* which prefers mildly alkaline sites, observed in Loch of Craiglush compared to Loch of Butterstone and Loch of Lowes. The lower abundance of *N. flexilis* in Loch of Craiglush may have made it more vulnerable to competition from *Elodea* spp., particularly given its annual reliance on seed production and dispersal. The "Allee effect" describes the relationship between population growth rates and population density in sparse populations, and implies that, in small populations reliant on sexual reproduction, small decreases in population density (for example, as a

result of increased competition from invasive species) can lead to drastic reductions in the population density of the following generation (Drake, 2004). This may have been further exacerbated by the decline of *N. flexilis* in Loch of Butterstone and Loch of Lowes, which, given the fact that *N. flexilis* seeds disperse over relatively large distances, could have further reduced the supply of *N. flexilis* seeds to Loch of Craiglush (Wingfield *et al.*, 2004). However, because neither seed production, seed movements nor *Elodea* abundance are represented in either the monitoring or the paleolimnological records, further research is required to test this hypothesis.

6.3.8 What lessons about *N. flexilis* can be learned from paleoecology*?*

The research presented in chapter 5 demonstrated that *N. flexilis* seeds are highly likely to be found in number in sediment cores taken from any location at a site where the plant is present. It also presented a framework for assessing the likely abundance of *N. flexilis* in a lake based on the number of seeds found in the sediment. This is further supported by the fact that *N. flexilis* seeds were found in all of the sediment cores presented in this chapter. Like other paleoecological studies involving *N. flexilis*, seed preservation in the sediments was good (Rasmussen and Anderson, 2005; Gałka *et al.*, 2012; Rūtiņa *et al.*, 2012). Where data was available (e.g. The Dunkeld-Blairgowrie Lochs in the 1990s - 2000s), the number of seeds found broadly corresponded with the abundance of the plant observed historically. In the dated core ESTH9 and the top portion of CRAIB, sections of the cores in which *N. flexilis* seeds were absent aligned with the documented disappearances of *N. flexilis* within a reasonable margin of error. The exceptions are a) seeds found in sediments dated from the 2000s in ESTH9, which likely represent failed attempts to reintroduce *N. flexilis* to Esthwaite Water, and b) seeds found in sediments dating from ~2007 in CRAIB, which, when compared to the results of intensive SCUBA surveys, create an unexplained "false positive" effect similar to that found at Loch of Butterstone in chapter 3. Despite these minor indiscrepancies, the evidence presented here strongly suggests that *N. flexilis* is ideally suited to paleoecological study at sites where historical monitoring data is lacking. In both of the examples studied here, paleoecology has provided insight into the reference conditions with which *N. flexilis* was

associated, and the timing and potential causes of the decline of the species.

Like chapter 4, the paleoecological results presented here support the notion that *N. flexilis* is able to grow within a range of different macrophyte communities based on the properties of the individual waterbody. Of the sites studied, *N. flexilis* was historically most abundant at Esthwaite Water, where it grew alongside a flora typical of mildly alkaline conditions, and least abundant at Loch of Craiglush, where it grew among more acid-tolerant species. These differing conditions subject it to different environmental pressures. At Esthwaite Water, the decline of *N. flexilis* was clearly linked to other ecological indicators of eutrophication; as an obligate CO2 user, *N. flexilis* is vulnerable to changes in organic carbon speciation that result from increased photosynthesis following nutrient enrichment (Wingfield *et al.*, 2006). At Loch of Craiglush, *N. flexilis* abundance was naturally lower due to lower pH, which affects the ability of the plant to reproduce (Titus and Hoover, 1991; 1993). This low abundance likely made it more difficult for the plant to persist when potentially faced with competition from *Elodea* spp., and a reduction in the supply of seeds as the species disappeared from neighboring, hydrologically-connected lochs. At Loch of Butterstone and Loch of Lowes, which had reference conditions similar to Esthwaite Water in terms of both macrophyte and diatom flora, there is evidence that mild eutrophication prevent the growth of *N. flexilis,* possibly in tandem with *Elodea canadensis* invasion.

The timing of the decline of *N. flexilis* is an important consideration in future conservation efforts. Following measured recoveries of total phosphorus, chlorophyll-*a* and Secchi depth at Esthwaite Water since 2000, attempts have been made to reintroduce *N. flexilis* at the site from seed (JNCC, 2012). However, the paleoecological evidence presented here shows that the decline of *N. flexilis* from peak abundance occurred much earlier than is indicated by the historical records, and that the plant was historically associated with significantly less nutrient rich, less alkaline and clearer waters than are present currently. This insight suggests that further recovery of Esthwaite Water is necessary before the species can be reintroduced. Such insight could not be gained from historical records alone.

6.4 Conclusions

Where data was available, macrofossil reconstructions of *N. flexilis* population change at Esthwaite Water and Loch of Craiglush were faithful to population changes recorded in historical botanical datasets. Differences in *N. flexilis* seed abundances in the cores highlight the fact that *N. flexilis* is more abundant in some lake types than others. Esthwaite Water and Loch of Butterstone formerly supported abundant populations of *N. flexilis*, in association with *Chara* spp. and *Nitella* spp., and Cladocera and diatom assemblages typical of clear, mildly-alkaline conditions. In contrast, Loch of Craiglush supported neither *N. flexilis* nor the charophytes typically associated with mildly alkaline conditions in abundance. Rather, its reference community was characterised by acid-tolerant *Potamogeton* spp., Cladocera and diatoms. At Esthwaite Water, the loss of *N. flexilis* in the 1980s was a result of long-term eutrophication. In the initial stages of eutrophication, from ~1915, *N. flexilis* populations declined, likely due to inhibited carbon fixation as increased photosynthesis in the lake led to reductions in dissolved CO_2. However, the species persisted for ~60 years until, in the 1980s, inputs of nutrients from the Hawkshead treatment works led to reduced light availability in the lower portions of the photic zone, and *N. flexilis* disappeared. At the Dunkeld-Blairgowrie lochs, this process may have been expedited by the expansion of *Elodea* spp., which, favouring elevated nutrient levels and using bicarbonate to retain growth rates when CO_2 levels reduced, might have out-competed *N. flexilis* in silty habitats in the lower portion of the photic zone. Loch of Craiglush was isolated from the major sources of nutrients in the catchment, and the cause of the decline of *N. flexilis* at this site remains uncertain. It is hypothesised that the small populations of *N. flexilis* at Loch of Craiglush were likely unable to maintain carrying capacity when faced with competition from *Elodea* spp. and the loss of *N. flexilis* from all hydrologically connected sites, but more research is required to test this theory.

7. Lessons learned, and recommendations for the conservation of *N. flexilis* in the UK

7.1 Introduction

The aim of this study was to use contemporary ecology in combination with paleoecology to help provide an evidence-base for the conservation of *N. flexilis* in the UK. By supplementing the results of modern, geographically-widespread monitoring programs with insights on longer-term trends in *N. flexilis* population decline obtained from the sediment record, this study affords insights into the complex set of environmental interactions that influence the realized niche of *N. flexilis* in the UK. SCM was first implemented in 2000, and is a valuable resource for assessing the nature of *N. flexilis* habitat in lakes that are subject to the program. However, in terms of monitoring *N. flexilis* populations directly, SCM lacks a strategic and reliable approach. A solution to this problem is presented in chapter 3 in the form of a snorkel survey protocol that can be easily conducted and analysed alongside existing SCM methodologies.

Notwithstanding methodological inconsistencies, data from SCM surveys since 2000 show that *N. flexilis* grows in association with different vegetative communities in lakes with differing chemical characteristics (chapter 4). Furthermore, by influencing the nature of competition from other plant species, the water chemistry of a lake also influences the water depth and abundance at which *N. flexilis* grows.

In chapter 5, it was determined that *N. flexilis* is able to distribute seeds across an entire lake basin, making it suited to paleoecological reconstruction from a small number of sediment cores - a technique that is unusual in its application to a single aquatic macrophyte species (as opposed to an entire vegetative community). Chapter 6 shows that the influence of water chemistry on *N. flexilis* abundance and resilience to ecological change is evident in sediment cores taken from sites at which *N. flexilis* has disappeared over the past century. At Esthwaite Water, *N. flexilis* was originally associated with a community characterised by charophytes and *Isoetes lacustris* - species typically

associated with clear, oligo-mesotrophic, mildly alkaline lakes. *N. flexilis* declined markedly in response to the early stages of nutrient enrichment in the 1910s, but the plant apparently persisted at lower abundances for several decades before eventually disappearing as nutrient enrichment intensified following discharge from the Hawkshead Wastewater Treatment Works in the 1980s. At the Dunkeld-Blairgowrie Lochs, *N. flexilis* was originally less abundant and associated with *Potamogeton* spp typically representative of mesotrophic, circumneutral lakes with slightly lower water transparency compared to Esthwaite Water. There was evidence for mild eutrophication at these lochs from agricultural inputs and fish cages, however changes in diatom, macrofossil and Cladocera communities were not as marked as in Esthwaite Water. Despite this, the disappearance of *N. flexilis* from the lakes was sudden, indicating that, within the *Potamogeton*-rich communities, *N. flexilis* is less resilient to ecological change than it is within *Chara*- and *Isoetes*- rich community. By drawing on the results of the contemporary ecological surveys, it was possible to reveal potential mechanisms for the differences between sites (for example, the vulnerability of *N. flexilis* to competition in different light conditions), and the paleoecological studies demonstrated examples of different scenarios of *N. flexilis* population change in sites with different chemical and biological characteristics. Thus, this study highlights the benefits of combining contemporary ecology and paleoecology for informing conservation strategies for rare species.

7.2 The current status of *N. flexilis* in the British Isles

Alongside Sweden, the British Isles is home to one of the largest populations of *N. flexilis* in Europe (Lansdown, 2011). Because of its European importance, the plant attracts the highest levels of UK protection, both as a Schedule 8 species in the Wildlife and Countryside Act and as a priority species within the UK Biodiversity Action Plan (HMSO, 1994). Since it was first recorded by Daniel Oliver in Cregduff Lough, Galway, Ireland in 1850 (Oliver, 1851), the number of sites at which the status of *N. flexilis* is known has increased with increased survey effort. Through collation of historical botanical records, this study identified four main survey periods. The first, in the 1920s

and early 1930s, aligns with the activities of eminent botanists such as Pearsall (e.g. Pearsall, 1915). The second reflects a period of increased freshwater botanical interest following the disappearance of *N. flexilis* from England in the late 1980s (e.g. James and Barclay, 1996). The third represents the PhD research of Wingfield (2002). Finally, the introduction of SCM in 2000 has led to an increase in the number of sites surveyed for *N. flexilis* in the 21st century; it is these SCM results that formed the basis of the contemporary ecological work conducted in this PhD. In Wingfield's PhD thesis, concern was raised for *N. flexilis* because it was found to have become extinct at nine former Scottish and Irish *N. flexilis* sites, most of which were located in agricultural catchments (e.g. Loch Marlee in Perthshire and Lough Nafeakle in Galway) (Wingfield, 2000). Since 2002, the number of former *N. flexilis* sites at which the plant was absent from surveys conducted during the last ten years has increased to 32; 15 in Ireland, 15 in Scotland and two in England. The sites at which *N. flexilis* is absent are no longer limited to agricultural catchments. *N. flexilis* is now absent from four sites in the Western Isles; it was last seen in Loch Bun an Ligidh, West Loch Ollay and an unnamed loch on South Uist in the 2000s, and in Loch Grogary in 2010. The Scottish Islands have previously been indicated as relatively un-impacted by human activity (McCloskey and Spalding, 1989), but it is clear that *N. flexilis* populations are now in decline even in these remote regions.

The declining population of *N. flexilis* in the UK echoes similar patterns in Europe and globally. According to the International Union for Conservation of Nature (IUCN) Red Data Book, *N. flexilis* is classified as Regionally Extinct in Switzerland and Poland and Critically Endangered in Germany, Sweden and Belarus (Lansdown, 2011). Behind the UK and Ireland, Sweden has the largest known population of *N. flexilis* in Europe, but the plant is currently only found in four Swedish lakes, having apparently disappeared from a further six sites over the past 100 years (Edqvist, 2006). In the USA, where *N. flexilis* is more common, changes in the distribution of the species have also been observed; in Ohio, *N. flexilis* was considered to be a common species at the turn of the 19th/20th century, but, between 1950 and 1970, it was collected from only 12 locations in the state (Wentz and Stuckey, 1971).

Although it is clear that the UK population of *N. flexilis* is in decline, there remain some sites which are known to have previously contained the species at which the current status of the plant is unknown. A number of these sites are impossible to re-visit because of a lack of clarity on their location in the historical botanical records. For example, records exist labelled "Near River Tay", "Benbecula Aerodrome", and "Pond near Manchester", all quoted with, at best, a four-figure grid reference. There has also been some discrepancy in past estimates of the number of *N. flexilis* sites due to duplicate records resulting from single sites that have been given multiple names, for example Loch Scaraidh/Scarie Loch, and Loch an Taigh-Sgoile/Schoolhouse Loch. A further problem in analysing historical botanical records is the fact that "positive" records (i.e. surveys during which *N. flexilis* was recorded) are more readily available and easy to find than "negative" records (i.e. surveys during which *N. flexilis* was absent). Although best efforts have been made to remove duplications and to include negative records, the accuracy of the database presented with this PhD cannot be guaranteed without in-depth local knowledge of individual sites. Taking into account these uncertainties, it is estimated that, of the 41 sites in the British Isles at which the status of *N. flexilis* is currently unknown, the locations of 30 (5 in Scotland and 25 in Ireland, listed in Appendix 9) are known and should be surveyed for *N. flexilis* in order to fill in the gaps in the data.

7.3 Techniques for monitoring changing populations of *N. flexilis*

Historical botanical records are largely the result of individual surveys, each with a different scientific aim and survey approach. Although standard techniques for aquatic plant surveys, for example the use of bathyscopes and grapnels, have existed since Pearsall's work in the Lake District in the 1910s (Pearsall, 1920), sampling strategy and effort has varied across different studies. It wasn't until the introduction of SCM in 2000 that widespread surveys of aquatic macrophytes using standard methods were adopted. Because of their standard methodologies, SCM surveys from different sites and different years can be compared to each other statistically. Chapter 4 of this PhD demonstrates the

potential for SCM datasets to reveal important ecological information that cannot be obtained from smaller-scale studies, for example the differences in vegetative communities associated with *N. flexilis* in different lakes. However, the SCM methods are largely based around the use of grapnels and bathyscopes. Several studies have indicated that, for aquatic plants living in deep water, snorkel or SCUBA surveys may be more appropriate (e.g. Sheldon and Boylen, 1978; Wade and Bowles, 1981). *N. flexilis*, specifically, has been shown to be more frequently recorded using SCUBA at one site in the USA, for example (Capers, 2000). The evidence presented in this study suggests that, in Scotland, grapnel surveys under-record *N. flexilis* compared to in-water surveys, particularly at water depths below ~1.5m. Furthermore, it demonstrated that in-water survey techniques were able to provide additional qualitative information on the condition of *N. flexilis,* for example at Glenastle Loch, where filamentous algae were observed to be smothering *N. flexilis* plants. Although SCUBA surveys are expensive, take considerable time and require equipment that is not easy to transport, snorkel surveys provide the option to use in-water techniques in a cost- and time- effective manner. In light of these results and existing evidence in support of in-water survey techniques, this study suggests that snorkels should be used wherever possible. To this end, a snorkel-based sampling strategy based around existing SCM protocols is recommended - the full details are outlined in chapter 3. This strategy is based around a fixed geographical location that is temporally consistent, ensuring that surveys are comparable through time. By consistently surveying in the same place, *N. flexilis* populations can be examined with relation to a relatively fixed morphological habitat, and any changes through time can be more accurately attributed to environmental changes. This protocol could easily be applied to other rare deep-water aquatic species, and multiple target species could be assessed in this way in a single survey.

Whilst snorkel surveys are suitable for assessing the extent of existing populations of *N. flexilis,* it is not practical to conduct all aquatic macrophyte surveys in this way. This may mean that new or previously unknown *N. flexilis* sites could remain undetected, especially if the plant is growing at greater depths than those typically surveyed during SCM. This is particularly true for sites where boat transects are deemed unsuitable; a

decision which should not be taken lightly in the light of this research. An example is Loch Phuirt Ridh, which was added to the 2016 SCM snorkel surveys after a small fragment of *N. flexilis* was found on a grapnel during a separate survey. The site was not previously known to contain *N. flexilis,* but the snorkel survey eventually revealed that the plant was relatively abundant, growing in 25% of sample points. The recent development of eDNA techniques has the potential to rapidly detect the presence of *N. flexilis* in sites at which it has not previously been found. *N. flexilis* is one of seven aquatic macrophytes for which sequencing of the complete chloroplast genome has been achieved (Peredo *et al.*, 2013; Hu *et al.*, 2017), and primers are currently being tested for quantitative polymerase chain reaction (qPCR) (N. Crutchley, pers. comms.). The aim is to extract these primers from both water and sediment samples, allowing the presence of *N. flexilis* in any lake to be easily and quickly detected. This could then be carried out both by professional ecologists and potentially by citizen scientists, as tested on a large scale in the UK Great Crested Newt surveys in 2013 (Biggs *et al.*, 2014). However, because eDNA techniques can produce false-positives and false-negatives, detection of *N. flexilis* DNA in samples taken from new sites would need to be followed up by a survey to confirm the result. This study has shown that, particularly in cases where *N. flexilis* abundance is low, in-water survey techniques like snorkeling give the best chance of correctly identifying whether the plant is present or absent.

This PhD, in combination with the ongoing work on the detection of *N. flexilis* eDNA, represents a significant advance in the tools available to directly monitor populations of *N. flexilis*. However, whilst knowledge on the location and abundance of a target species is valuable, the value of the conclusions drawn about the ecology of *N. flexilis* is limited by the paucity of available information on the water chemistry of the study sites. Macrophyte surveys were conducted only during the summer season when primary productivity is highest, but water chemistry is both diurnally and seasonally variable. Thus, water chemistry variables measured on site represented only a snapshot of the conditions at the time of the survey, rather than capturing the full range of environmental conditions that may influence *N. flexilis* growth. Because of this, secondary water chemistry data from the SEPA monitoring program was used in the ecological analyses in

chapter 4. For most sites, this consisted of between one and eight water samples collected from the centre of the lake each year. Whilst this sampling frequency is adequate for detecting changing trends in water quality at individual sites over a period of several years, it is not adequate for capturing the full range of environmental conditions present in any single growing season. Furthermore, the different sampling frequencies and timings at different sites meant that data were not comparable across sites. It was therefore necessary to take the annual mean of measured water chemistry at each site, but this reduced the number of water chemistry samples available that corresponded to each individual SCM survey to one. This was not enough to perform meaningful statistical comparisons of water chemistry at different sites. Furthermore, there were some sites for which no water quality data were available (e.g. West Loch Ollay), and some sites at which water quality was monitored at the river inflows/outflows rather than in the lake itself and was therefore not directly comparable to the rest of the data in terms of sample collection methodology (e.g. The Dunkeld-Blairgowrie Lochs). Despite the limited number of directly comparable data points on water chemistry in this study, the differences in water chemistry that were identified between the sites were consistent with differences in the vegetative communities present. For example, lakes with the highest measured mean annual alkalinities were characterized by *Chara* and *Potamogeton* spp considered in the Plant Lake Ecotype Index (PLEX, a tool used for assessments of UK lake typology) as typical of lowland, base-rich lakes (Duigan *et al.*, 2006). The conclusions on the links between *N. flexilis* and water chemistry presented here are based on the assumption that the data are representative of the nature of the sites in question, however it is clear that future studies that compare SCM data with water chemistry data would benefit from an improved monitoring scheme in which water chemistry samples are consistently taken at the same frequency, at the same time of year and using the same methodologies at all sites.

7.4 Threats to *N. flexilis* in the UK

Following the PhD thesis of Wingfield (2002), several hypotheses have been put forward for the decline in *N. flexilis* in the UK, including acidification, eutrophication and

competition from aquatic invasive plants (Wingfield *et al.*, 2004). TWINSPAN analysis of SCM data presented in chapter 4 shows that *N. flexilis* presents differently in different environmental conditions, and suggests that the vulnerability of the plant to each of these proposed threats is dependent upon the characteristics of the site in question in each individual case. The paleoecological work in chapter 6 supports this by providing empirical evidence that, at two sites in agricultural catchments, eutrophication over a decadal timescale has contributed to the extinction of *N. flexilis*.

7.4.1 Acidification

When the results of all SCM surveys were considered, this study found that *N. flexilis* was generally more abundant at sites with relatively low pH (< ~7.5). This result is unexpected because, in laboratory experiments, *N. flexilis* has been shown to be unable to reproduce in waters with a low pH (Titus and Hoover, 1993). None of the sites included in this PhD study had a measured pH < 6.5, so the lower end of the tolerance range of *N. flexilis,* which is believed to be between 5 and 6, was not observed (Titus and Hoover, 1993). However, there were some sites where *N. flexilis* was absent or present in very low abundance where pH was between 6 and 7, such as Loch an t-Sagairt and Loch Bun an Ligidh. These sites were characterised by acid-tolerant floras, including *Juncus bulbosus* and *Lobelia dortmana.* Relative to most *N. flexilis* sites, the catchments of these sites have a more upland situation and are less commonly used for agriculture - Loch an t-Sagairt, for example, is 50m above sea level (most other *N. flexilis* lakes on the Inner Isles are situated below 10m a.s.l), and it's catchment is comprised of 90% open shrub heath and unimproved grassland, and 10% inland water (Fuller *et al.*, 2002). At such sites, *N. flexilis* is (or likely was historically) surviving outside of it's preferred ecological niche, and is thus particularly susceptible to future changes that could lead to a decrease in pH. All of these sites are situated in regions where it is considered highly likely that future acid deposition will exceed acid buffering capacity (Kernan *et al.*, 2004).

Although this study was not able to produce empirical evidence of a case of *N. flexilis* disappearing due to acidification in the UK, paleoecological evidence from Loch of

Craiglush does suggest that the relatively acidic conditions at the site made it historically less favorable to *N. flexilis* than it's more base-rich neighbours. Of the three Dunkeld-Blairgowrie Lochs studied, Loch of Craiglush was identified as having a historically different flora to that of it's neighbouring lakes. In contrast to Loch of Butterstone, where typically circumneural to alkaline plants were present, the reference flora of Loch of Craiglush was characterised by a wide range of *Potamogeton* spp. in combination with acid-tolerant macrophytes like *Juncus bulbosus* and *Lobelia dortmana.* This is echoed in subfossil diatoms in sediment cores taken from the Dunkeld-Blairgowrie Lochs, in which the algal flora of Loch of Craiglush was found to contain more acid-tolerant species than the other sites (Finney, 1998). Contemporary water chemistry monitoring also shows that pH at Loch of Craiglush fluctuates at around 7 - usually between 0.5 and 1 lower than at Loch of Butterstone and Loch of Lowes. Although *N. flexilis* has been historically recorded at the site, macrofossil records reveal that the plant was likely only ever present at low abundances. There are periods of time during which *N. flexilis* was notably absent from the macrofossil record (e.g. c1879 - 1920), although it was not possible to determine from the ecological proxies whether this was a result of short-term fluctuations in pH. Research from elsewhere has demonstrated that the plant has been removed from sites by natural decreases in pH in cases of historical climate change: For example, a paleoecological study of a kettle hole located in the Rominicka Forest, Poland, showed that the disappearance of *N. flexilis* during the Subboreal corresponded with an increase in acid-tolerant *Sphagnum* spp., probably as a result of lake acidification triggered by the expansion of *Picea abies* in the catchment following climate cooling and drought (Gałka *et al.*, 2012). A similar pattern of *N. flexilis* decline linked to acidification in the Subboreal has also been identified in a sediment core taken from Lake Piļvelu, Latvia (Rūtiņa *et al.*, 2012). However, there is no contemporary evidence for extinction of *N. flexilis* as a direct result of acidification. It has been observed that, in both Sweden and Scotland, acid-intolerant macrophyte species, most notably *Myriophyllum alterniflorum,* have widely persisted in contemporary cases of freshwater acidity, although the reasons for this are not understood (Arts, 2002). It seems that, despite evidence suggesting that *N. flexilis* populations in some UK lakes should be threatened by acidification, this threat has not yet been realised. Nevertheless, continued monitoring of the more acidic *N.*

flexilis lakes is recommended.

7.4.2 Eutrophication

Although measured nutrient concentrations at all sites in this study were low, total phosphorus concentrations were significantly higher in lakes where *N. flexilis* was absent. Indeed, many of the former *N. flexilis* sites on the Scottish mainland where the plant was not present in the most recent survey were characterised by relatively high phosphorus concentrations and a flora typical of mesotrophic conditions, for example the Dunkeld-Blairgowrie Lochs. This echoes the findings of Wingfield (2002), who also noted a significantly higher phosphate concentrations in lakes without *N. flexilis*. In fact, there are indications that nitrogen may play a stronger limiting role on *N. flexilis* growth than phosphorus (e.g. Moeller *et al.*, 1988; Tracy *et al.*, 2003), and, in this study, both *N. flexilis* presence and higher abundances of *N. flexilis* were found to be associated with higher concentrations of nitrates in the water. It is widely quoted that the general mechanism for macrophyte decline in eutrophicated lakes is the reduction of light availability for photosynthesis as a result of shading, either by phytoplankton or by epiphytes (Phillips *et al.*, 1978). However, the results of this study suggest that *N. flexilis* thrives in low light conditions; sites where *N. flexilis* was most abundant generally had comparatively low LECs, and, at these sites, *N. flexilis* was growing at water depths of up to 2.5m. Furthermore, this study showed that *N. flexilis* has a preference for fine, silty sediments, as often promoted by the decay of phytoplankton and other organic matter prevalent in more eutrophic systems (Schutten *et al.*, 2005). An alternative mechanism for the apparent lack of *N. flexilis* at sites with higher phosphorus concentrations and more typically mesotrophic floras must therefore be found.

In lakes with a circumneutral pH, the process of eutrophication is often coupled with alkalinisation (Arts, 2002). As photosynthesic rates increase following nutrient enrichment, more carbon dioxide is consumed by plants and the ratio of of HCO3- to CO2 increases, leading to an increase in pH (Talling, 1976; 1985). This study has shown that *N. flexilis* is more abundant in waters with a higher pH, and Wingfield (2000) also

showed lakes containing *N. flexilis* to have significantly higher alkalinities and concentrations of calcium and magnesium than lakes without *N. flexilis*. However, *N. flexilis* is an obligate carbon dioxide user and, unlike many plants found in hard water environments, it cannot use bicarbonate for photosynthesis (Hough and Wetzel, 1978; Hough and Fornwall, 1988). This study reveals that, in lakes with high measured alkalinities and floras typical of base-rich conditions (e.g. Loch Grogary), *N. flexilis* is typically found in the deepest portion of the photic zone, away from other plants. This suggests that, despite its preference for moderately alkaline waters, *N. flexilis* cannot compete with bicarbonate-using macrophytes in situations where the dominant form of available carbon is HCO3- and it and must therefore occupy zones of lower light penetration in deeper water. In more acidic lakes, *N. flexilis* does not grow as abundantly, but, because the dominant form of available carbon is more likely to be CO2, it can compete with other macrophytes and grows in a wider range of depth zones. Naturally base-rich lakes tend to be associated with clear waters and relatively high light penetration, even at considerable depth. Where increased alkalinities exist as a result of eutrophication, water clarity is likely to be compromised and competition from other plants increased as excess phosphorus increases primary productivity, thus preventing *N. flexilis* from retreating into deeper waters and away from other, bicarbonate-utilising macrophytes as it would in a naturally base-rich site. Therefore, although *N. flexilis* is able to survive at low abundances in naturally base-rich environments with low productivity, it is susceptible to alkalinisation coupled with eutrophication in circumneutral and mildly alkaline lakes.

The paleoecological results of this PhD (chapter 6) provide evidence for the impacts of eutrophication on circumneutral/mildly alkaline lakes in the UK. The history of eutrophication at Esthwaite Water has been well documented in both long-term chemical monitoring and paleoecological reconstructions using diatoms and algal pigments (Dong *et al.*, 2011; Dong *et al.*, 2012; Moorhouse, 2016; Maberly *et al.*, 2017). Anthropogenic nutrient enrichment began in Esthwaite Water in ~1915, when algal communities began to shift as a result of catchment land use changes including the intensification of agriculture and increases in tourist traffic and associated infrastructure (Dong, 2011;

Moorhouse, 2016). The first documented macrophyte records from Esthwaite Water date from a shoreline survey conducted in 1914, and the first full macrophyte survey followed in 1917 (Pearsall, 1920). There are therefore no macrophyte records that pre-date the initial eutrophication of Esthwaite Water. The macrofossil analysis presented in this study shows that, pre-1915, the flora of Esthwaite Water was characterised by high abundances of *N. flexilis* in association with *Chara* spp., *Nitella* spp., and *Isoetes lacustris.* The results of the SCM surveys presented in chapter 4 show that this flora is typical of *N. flexilis* sites that are clear and oligo-mesotrophic, with a circumneutral to mildly alkaline pH. Because of the high water clarity typically associated with these sites (confirmed by the presence of the Cladoceran *Camptocercus rectirostris* in this portion of the sediment core as well as monitoring records of Secchi depths of 3 - 4m), *N. flexilis* would have been able to retreat into deeper water as carbon dioxide was consumed by photosynthesis in shallower areas, away from the increasing biomass of bicarbonate-utilising plants and epiphytic algae, which all increased in the paleoecological record from ~1915). This is reflected by the fact that, despite a marked decrease in the abundance of *N. flexilis* remains in the sediment record from ~1915 onwards, the species apparently persisted for another ~60 years. In the 1980s, discharges from the Hawkshead Wastewater Treatment Works in combination with nutrient inputs from new fish cages in the lake led to an intensification of nutrient enrichment of Esthwaite Water, causing an increase in planktonic diatom species and floating leaved macrophytes and a decrease in measured Secchi depth to a low of ~2m (Dong *et al.*, 2011; Dong *et al.*, 2012; Moorhouse, 2016; Maberly *et al.*, 2017).

Monitoring records of pH, temperature and alkalinity from 1993 confirm that HCO_{3^-} was the dominant form of organic carbon in the lake during the *N. flexilis* growing season at this time (Maberly, 1996). At this point, it is likely that *N. flexilis* was unable to survive the combination of low availability of carbon dioxide for photosynthesis in the majority of the photic zone and low availability of light in the deeper waters away from bicarbonate-utilizing species. Similar patterns of *N. flexilis* movement to deeper water before eventual loss of the species are implied by combining historical chemical and macrophyte monitoring data with diatom reconstructions at Loch of Lowes and Loch of

Butterstone in Perthshire. However, the macrofossil data from these sites not only pre-date the most significant period of nutrient enrichment but also are insufficiently resolved, and collection of further littoral cores from these sites is required before reliable conclusions on the decline of *N. flexilis* can be drawn.

7.4.3 Competition from invasive aquatic plants

Elodea canadensis and *Elodea nuttallii* are native to the Americas, but have been introduced to the UK, the former as a fragment on a log imported from Canada in 1836 and the latter from an unknown source in 1966 (Simpson, 1984). Both species have caused widespread problems since their introductions, including blocking sluices and drains, raising water levels, and entangling swimmers (Simpson, 1984). The primary concern, however, is their ability to vegetatively reproduce rapidly, excluding native macrophytes like *N. flexilis* through competition and shading (Simpson, 1984; Nichols and Shaw, 1986; Bowmer *et al.*, 1995).

12 of the 26 current and former *N. flexilis* sites regularly surveyed by SCM currently contain one of the two aquatic invasive species *Elodea canadensis* and *Elodea nuttallii.* Like *N. flexilis*, *Elodea* spp. were found in association with plant communities ranging from acid-tolerant to base-rich, with *Elodea nuttallii* replacing *Elodea canadensis* in the most alkaline environments. Although a negative correlation was found between *N. flexilis* percentage cover and *Elodea* percentage cover, and *Elodea* spp. were shown to occupy the same depth zone and silty substrates as *N. flexilis,* there were many sites at which both species seemed to co-exist within the same micro-habitat. Dense stands of *Elodea nuttallii* were observed at Loch Scaraidh, but there were also large areas of bare substrate present within the *N. flexilis* depth zone, suggesting that competition from *Elodea nuttallii* was not solely responsible for the absence of *N. flexilis.* Given the absence of long-term records of *Elodea* abundance at many *N. flexilis* sites, it is impossible to know whether dense stands may develop at sites at which the two species co-exist, or whether existing stands may collapse and allow new *N. flexilis* growth to occur.

Like *N. flexilis*, *Elodea* spp. favour waters with above neutral pH, although *Elodea* spp., particularly *Elodea nuttallii*, appear to be more tolerant of high alkalinity than *N. flexilis.* *Elodea* spp. have repeatedly been shown to be extremely effective at using bicarbonate for photosynthesis (Allen and Spence, 1981; Sand-Jensen and Gordon, 1986). Furthermore, *Elodea* spp. are able to tolerate unusually low light conditions through phenotypic plasticity; individual plants respond to low light levels by producing taller stems with longer leaves (Simpson, 1988). This means that, theoretically, *N. flexilis* is at greatest risk from competition from *Elodea* spp. at the more base-rich end of its realised niche, where it usually thrives in low light conditions away from other plants. Reduced CO_2 availability in the deeper edges of the photic zone (3-4m) following invasion by *Elodea canadensis* is thought to be the main reason for the dramatic decline in *N. flexilis* observed since the 1980s in Lake Steinsfjord, Norway (Mjelde *et al.*, 2012), however there is no evidence for similar declines in *N. flexilis* at sites where *Elodea* spp. are present as the sole anthropogenic influence in Scotland. *Elodea canadensis* is hypothesised to have drastically reduced the extent of *N. flexilis* at Tangy Loch, but this study was conducted after *Elodea canadensis* spread in Loch Tangy and it was therefore not possible to provide evidence to support this. It was noted that *Elodea canadensis* occupied ~75% of the sample points >1m depth, whilst *N. flexilis* was present in much shallower water than would otherwise be expected, with a minimum water depth of 0.08m.

In this study, *Elodea* spp. were found in abundance at former *N. flexilis* sites with elevated phosphorus concentrations (e.g. Loch of Lowes). This suggests that, in cases of nutrient enrichment, *Elodea* spp. are able to survive the combination of low light penetration and increased ratios of HCO_{3-} to CO_2 where *N. flexilis* cannot. As shown in Fig. 7.1, the combination of eutrophication and *Elodea* spp. invasion may therefore be detrimental for *N. flexilis*. Whilst the paleoecological study revealed that the extinction of *N. flexilis* following nutrient enrichment at Esthwaite Water occurred over a period of several decades, the plant seems to have disappeared much more suddenly from the Dunkeld-Blairgowrie Lochs. Although contemporary monitoring records at the lakes show increased nutrient concentrations, summer algal blooms and reduced Secchi depths

at Loch of Butterstone and Loch of Lowes, ecological indicators suggest that neither benthic diatom communities nor macrophyte communities have reached the level of species turnover exhibited in the cores taken from Esthwaite Water. Rather, the uppermost portions of the cores from Loch of Butterstone and Loch of Lowes more closely resemble the communities found at Esthwaite Water during the early stages of eutrophication in the ~1910s, when *N. flexilis* declined in abundance but nevertheless persisted. However, extensive contemporary snorkel and SCUBA surveys of the Dunkeld-Blairgowrie Lochs have documented the extinction of *N. flexilis* at the sites between 2004 and 2007 (Benthic Solutions, 2007). This extinction was preceded by the retreat of the plant to deeper waters, as expected in cases of nutrient enrichment; in 2007, *N. flexilis* was only growing in Loch of Lowes in water depths below 1.5 m (SNH, 2007). The extinction of *N. flexilis* from these deep water zones coincides with a rapid expansion of *Elodea* spp. in the lochs in the mid-late 2000s (SNH, 2007). It is likely that, in these lochs, competition from *Elodea* spp. has expedited the loss of *N. flexilis* in the face of nutrient enrichment.

7.4.4 Climate Change

Whilst acidification, eutrophication and alkalinisation, and aquatic invasive plants all emerged as potential threats to *N. flexilis* in Scotland through this PhD study, the impacts of climate change were difficult to isolate from other, more deleterious influences at the sites researched. Nevertheless, the observations made allow hypotheses on the fate of the plant under climate change to be suggested for further investigation. Chapter 4 shows that, in the UK, the majority of lakes in which *N. flexilis* has become extinct over the last fifty years are located in England, mainland Scotland and southern Ireland. This could be attributed to the fact that these areas are the most intensively farmed and densely populated of all regions in which *N. flexilis* occurs, however it is also notable that most of these locations are at the southerly limit of the plant's UK distribution. This pattern is repeated in Europe, where the regions in which *N. flexilis* is thought to have become extinct over the past 100 years (Switzerland, Poland and Germany) also represent the southernmost limit of the plant's contemporary range (Lansdown, 2011). In Ohio, USA,

N. flexilis was observed to have shifted its observed range in 1900 to the north by the 1970s, to be replaced by the more southerly favouring species *Najas marina* and *Najas guadalupensis;* a shift that was attributed to a combination of increased turbidity and eutrophication in Ohio waters and a gradual climate warming in the state (Wentz and Stuckey, 1971).

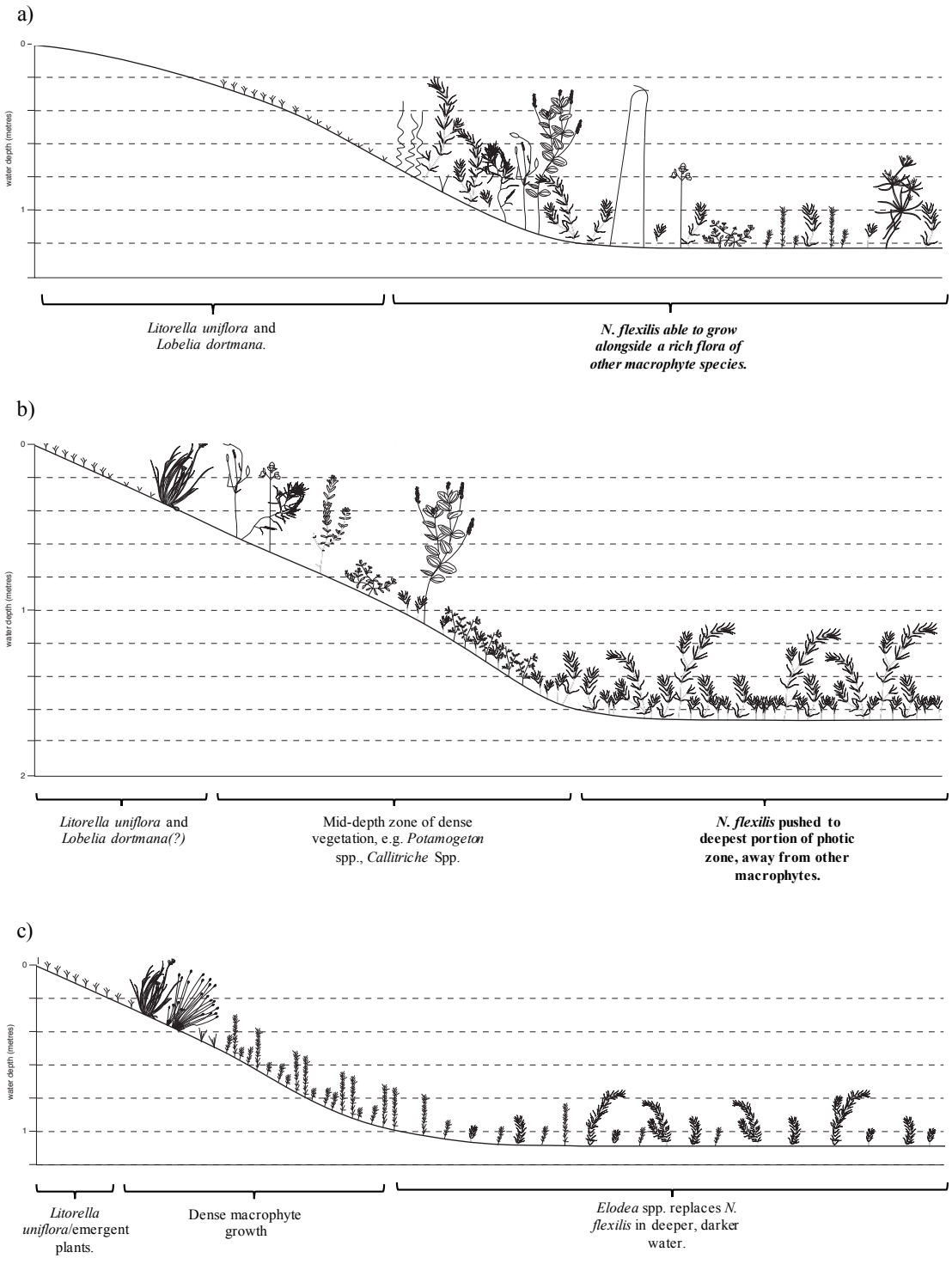

Figure 7.1 Conceptual progression from non-enriched lake (a), to enriched lake in which N. flexilis *can only photosynthesise in deep waters where CO2 is available (b), to enriched lake where* Elodea *spp., outcompete* N. flexilis *in deeper waters (c).*

Although the decline of *N. flexilis* in the more southerly regions of Europe has not been directly attributed to climate warming, many other aquatic species have already been observed to have shifted their distributions north in conjunction with contemporary climate change; in the UK, for example, historical botanical records reveal that 20 species of Odonata and 15 species of fish have shifted northward over a 40 year period (Hickling *et al.*, 2006), however, paleoecological records on a Holocene timescale show that sub-fossil seed fragments of *N. flexilis* are abundant in early sediments from throughout the UK (e.g. Hockham, Norfolk (Godwin and Tallintire, 1951), Nazeing, Essex (Allison *et al.*, 1952), Tregaron Bog, Wales (Godwin and Mitchell, 1938). This suggests that the plant was more widely distributed during the post-glacial climatic optimum (circa 7500 – 5000 B.P.) than currently, and that the plant's UK distribution declined in the mid-late Holocene because of climatic cooling (Pennington, 1974). Although this implies that *N. flexilis* is tolerant of warmer conditions than suggested by its contemporary northerly distribution, the species is not excluded from the influence of contemporary climate warming.

As freshwaters warm and the length of the growing season is extended, the primary productivity of aquatic ecosystems is likely to increase (Parmesan, 2006). This has the potential to cause the negative effects of eutrophication and alkalinisation on *N. flexilis* described above. Furthermore, the UK, and particularly western Scotland, is predicted to receive more rainfall, with an increase in the number of extreme rainfall events (Werritty, 2002). In many of the lakes in which *N. flexilis* is currently found, acid runoff from upland catchments is balanced by base-rich input from the underlying geology, creating clear, circumneutral or mildly alkaline lakes like those found on the Machair Sands of the Outer Hebrides. Any changes to rainfall patterns in these catchments have the potential to alter this balance; this study has shown that *N. flexilis* is particularly sensitive to changes in pH and alkalinity. Therefore, despite the ability of *N. flexilis* to survive in warmer temperatures, it is possible that the species will be vulnerable to the impacts of climate change through alterations to lake productivity and alkalinity. In order to fully understand the influence of contemporary climate warming on *N. flexilis*, long-term paleoecological investigations should be carried out on sites that are relatively isolated from

anthropogenic nutrient enrichment.

7.5 Recommendations for conserving *N. flexilis* in the UK

7.5.1 Protecting existing populations

This research presents the most comprehensive inventory of *N. flexilis* records in the UK to date. It is important that this knowledge on the location of existing *N. flexilis* populations is used to ensure that further losses of the species are avoided where possible. Because *N. flexilis* has protected status, many populations of the plant are now subject to regular monitoring, and the methods presented in chapter 3 will ensure that these efforts are reliable and comparable across time and space. Expansion of monitoring programs to include former *N. flexilis* sites that are not currently subject to SCM and any new sites identified through conventional methods or the use of eDNA will further improve our knowledge on the extent of the plant's distribution. However, in order to ensure protection of these existing populations of *N. flexilis,* it is not just the plant that needs to be monitored, but also any changes to its status or to the condition of its habitat that may pose a risk to the species. Wingfield *et al.* (2006) used TWINSPAN analysis to show that, in the UK, *N. flexilis* can be found growing as a component of several different plant communities. This study has shown that these different communities are representative of different environmental conditions, and that these environmental conditions define the most likely stressor or stressors that *N. flexilis* is likely to face. For example, *N. flexilis* is more likely to be at risk from competition from *Elodea nuttallii* invasion in an alkaline lake than in an acidic lake. By splitting *N. flexilis* sites into different "types" based upon their physical, chemical and biological attributes (as illustrated through the use of "divisions" in chapter 4), it is possible to assess the likely resilience of *N. flexilis* to the unique set of pressures faced in individual catchments. This allows informed decisions to be made on those *N. flexilis* habitats which should be prioritized, and the set of actions that should be taken in each case. An example of how the SCM sites currently containing *N. flexilis* may be prioritised, based upon the results of the TWINSPAN analysis conducted in chapter 4, is shown in table 7.1.

Table 7.1 N. flexilis *sites most likely to be at risk from different pressures. Sites marked with an x represent sites with high risk.*

Site	Low acid buffering capacity?	*Elodea* spp. present?
Division 2 – Most likely to be at risk acidification		
Loch Ballyhaugh		
Loch an T'Sagairt	x	
Loch Cuilc		
Loch a'Mhaddaidh	x	
Loch Nam Cnamh		
Loch Na Cuithe Moire		
Loch Gerraidh Mhic Iain		
Loch a Phuirt Ridh		
Schoolhouse Loch		
Loch Druidibeg	x	
Loch an Eilean		
Mid Loch Ollay		
Divisions 1 and 3 – Most likely to be at risk of eutrophication, with some risk from Elodea spp.*		
Loch Tangy		x
Loch Fada		x
Loch Glenastle		
Division 4 – Most likely to be at risk from Elodea invasion*		
Loch Scaraidh		X

*Many sites in these divisions no longer contain *N. flexilis,* and aren't listed here.
**Several sites in this division no longer contain *N. flexilis,* and aren't listed here.

7.5.2 Reversing environmental stressors

At sites at which *N. flexilis* has already become extinct, efforts should be focused upon identifying whether it is possible and worthwhile to restore the *N. flexilis* habitat, and, where relevant, carrying out positive actions with this intent. Whilst eutrophication appears to be having the greatest negative impact on *N. flexilis* populations in the UK (10

former *N. flexilis* sites in Scotland, for example, are currently experiencing nutrient enrichment), it is potentially the easiest threat to combat, since it is a relatively localised problem; nutrient sources generally originate from within the catchment of the affected site. Catchment management plans are increasingly common, and, allowing for delays caused by internal nutrient loading, are generally effective at reducing in-lake nutrient concentrations and chlorophyll *a* concentrations and increasing Secchi depths over a period of approximately 10-15 years (Jeppesen *et al.*, 2005). At Esthwaite Water, phosphorus loading is estimated to have fallen from 1677 kg TP annually in 1992-1993 to 747 kg TP annually in 2010, following the addition of tertiary treatment to Hawskhead wastewater treatment works and the closure of the fish farm (Maberly *et al.*, 2011). Chlorophyll *a* concentration and Secchi depth improved between 2000 and 2009, but ecological indicators of recovery are slower to respond and have not yet been observed (Maberly *et al.*, 2011b). Because most of the nutrients involved in the eutrophication of the Dunkeld-Blairgowrie Lochs comes from diffuse agricultural sources, it has been more difficult to mitigate, however the implementation of the Lunan Natural Care Scheme since 2004 has led to reductions in the concentrations of both phosphorus and nitrates observed at the Lochs. Whether these reductions have had a positive ecological influence remains unclear.

Invasion of lakes by *Elodea* spp. is common across the UK, however it is generally accepted that, after a few years of dominance, *Elodea* spp. populations at a newly invaded site will reduce and the plant will no longer be problematic (Simpson, 1984). However, this study implies that even small abundances of *Elodea* spp. have the potential to negatively impact *N. flexilis* population in situations where available CO_2 is limited. Physical removal of *Elodea* spp. is the most common control method, but is labour intensive and expensive, and needs to be carried out multiple times each season in order to limit the number of propagules available for overwintering (Newman and Duenas, 2010). This would need to be done manually since the use of machinery is precluded by the need to prevent damage to *N. flexilis* plants, however manual methods have been shown to leave ~10% of the plant untouched and are therefore ineffective (Soulsby, 1974). Because *Elodea* spp. are able to tolerate low light conditions, shading is not a

viable option (Abernethy *et al.*, 1996). The herbicide Diquat has been extensively tested on *Elodea* spp., and, where water is not turbid, can effectively eradicate the species, apparently with little impact on native species (Glomski *et al.*, 2005; Chisholm, 2007). The effect of Diquat on *N. flexilis* is unknown. The only known biological control for *Elodea* spp. is *Ctenopharyngodon inella* (grass carp) (Avault, 1965), but, notwithstanding the fact that grass carp are not native, the fish have been shown to graze on *Najas* species (Mitzner, 1978). Overall, there are few control mechanisms for *Elodea* spp., that guarantee minimal impact upon *N. flexilis* populations, and, as a result, management of *Elodea* spp., at *N. flexilis* sites in the UK has not been attempted (Vernon and Hamilton, 2011). Further research on the potential impacts on *N. flexilis* of new and existing physical, chemical and biological methods for the eradication of *Elodea* spp. is required before further action can be taken.

Global policies to reduce sulfur emissions, most notably the UN Convention on Long-Range Transboundary Air Pollution (CLRTAP) (1979), have made a significant impact in terms of addressing acidification over the last ~40 years. The UK Acid Waters Monitoring Network was established in 1988 to monitor ecological responses to emissions reductions, and has recorded improvements in pH, alkalinity and aluminum toxicity as well as some recovery of diatom, macroinvertebrate, macrophyte and salmonid fish communities at 11 acid-sensitive lakes across the UK over a period of 15 years (Monteith and Evans, 2005). However, these recoveries may not be a direct result of recovery from acidification, but rather a response to increases in dissolved organic carbon concentrations in lakes following changes in catchment use and global warming (Evans *et al.*, 2005). More recently, nitrogen deposition has been shown to play an important role in both acidification and nutrient enrichment in remote lakes with upland catchments (Maberly *et al.*, 2002, Curtis *et al.*, 2005), and recovery may be inhibited by the leaching of nitrates deposited in soils into surface waters (Curtis *et al.*, 2005). Sites located in coastal locations may also be subject to natural fluctuations in pH as a result of inputs of sea salt via soil retention and subsequent release (Evans *et al.*, 2001). Whilst *N. flexilis* is sensitive to reductions in pH, this study has shown that it may favour elevated nitrate concentrations and increases in dissolved organic carbon. It is clear that *N. flexilis*

is likely to be sensitive to changes in lake pH not only as a result of continued acid deposition, but also from changes in water chemistry in recovering waters as well as from natural fluctuations. Regular monitoring of water chemistry in *N. flexilis* sites sensitive to changes in pH is therefore recommended.

Whilst policies to reduce greenhouse gas emissions are in place (e.g. The UK Climate Change Act (2007); A UN Framework Convention on Climate Change (Kyoto Protocol) (1997)), it is clear that the impacts of global warming, including predicted range shifts and phenological changes in species of conservation interest, are likely to persist in the coming decades and centuries (IPCC, 2001). Aquatic systems are often thought of as a mosaic of isolated 'island' habitats within the terrestrial landscape, with ecological compositions dependent upon the ability of species to reach them via dispersal (Oertli *et al.*, 2002). In order to facilitate range shifts in aquatic organisms, a network of suitable aquatic systems across which the species is able to disperse must exist (Opdam and Wascher, 2004). However, over the past ~200 years, these networks have become increasingly fragmented both in the UK and mainland Europe as aquatic habitats have been either lost to drainage and infilling or damaged by pollution (Møller and Rørdam, 1985; Wood *et al.*, 2003; Dudgeon *et al.*, 2006). This habitat fragmentation has been shown to have a negative effect on a variety of aquatic species, including invertebrates, newts and other amphibians (Opdam *et al.*, 1993; Joly *et al.*, 2001; Cushman, 2006). Whilst this study has shown that, compared to many other aquatic plants, *N. flexilis* is able to disperse seeds over a relatively wide area, the "rafting" dispersal mechanism identified in chapter 5 is reliant upon hydrological connectivity. Furthermore, *N. flexilis* uses water currents to disperse pollen - a trait that is found in fewer than 5% of all aquatic species (Cox, 1988; Les, 1988; Philbrick and Les, 1996). Unusually for a plant exhibiting hydrophily, *N. flexilis* is unable to reproduce vegetatively and is therefore entirely reliant upon the dispersal of seeds (Hutchinson, 1975). Thus, *N. flexilis* is less able than most aquatic plant species to shift its range across a fragmented landscape as our climate changes. This is compounded by monitoring and conservation programs for *N. flexilis* that are centred around designated protected areas in fixed geographical locations; a fact that is highlighted in this study, which was only able to source extensive contemporary

data on *N. flexilis* from SCM surveys of SSSIs. In order to ensure the continued protection of *N. flexilis* as its range shifts, it is necessary not only to protect existing *N. flexilis* sites, but also to preserve and create hydrological connections to nearby healthy ecosystems suitable for the survival of the plant.

7.5.3 Reintroducting *N. flexilis*

Where former habitats have recovered to a state capable of supporting lost rare plant species, natural recolonisation may not occur if seeds are not able to disperse to the site. In such cases, reintroduction of individual plants is seen a necessary step for the conservation of rare species (Akeroyd and Wyse Jackson, 1995). In a review of the results of 249 plant reintroductions worldwide, Godefroid *et al.* (2011) found that success rates for such schemes are generally low, with only 16% of reintroductions resulting in fruiting. Successful reintroductions are generally undertaken using seedlings as opposed to seeds, which are introduced in large numbers and taken from multiple source habitats at which the species' populations are healthy (Menges, 2008; Godefroid *et al.*, 2011). Because *N. flexilis* is an annual plant incapable of vegetative reproduction, and has fragile above- and below-ground structures, Wingfield *et al.* (2004) argue that reintroductions should be made from seed rather than seedlings or adult plants. In laboratory experiments, up to 87% of tested *N. flexilis* seeds germinated uniformly and quickly, implying that reintroductions using seeds have the potential to be successful (Muenscher, 1936; Wetzel and McGregor, 1968; Wingfield, 2002). Genetic studies of UK populations of *N. flexilis* have not given any indication of the best location from which to source *N. flexilis* seeds for reintroduction, hence it is usually advised to take seeds from environments similar to the recipient lake (Wingfield *et al.*, 2004). To support such work in the future, chapter 4 identifies healthy populations of *N. flexilis* in the UK, and groups current and former *N. flexilis* sites according to their environmental conditions. Chapter 5 demonstrates that, if *N. flexilis* is present at a site, seeds are likely to be abundant in the sediment and thus readily available for translocation to another site. Based upon this knowledge, suitable and plentiful seeds for reintroductions can be collected from any site (or preferably multiple sites) within the same group or division as the recipient lake. The

paleoecological work in chapters 3 and 4 shows that *N. flexilis* seeds are also likely to be present in the seedbank of former *N. flexilis* sites. In cases where the extinction of the plant was relatively recent (e.g. Loch of Butterstone), this seedbank is easily collected using an Ekmann grab. Research on the excavation of former seed banks from infilled farmland ponds in Norfolk suggests that many aquatic macrophytes are able to germinate and grow once exposed to the right environmental conditions after 50 - 150 years of dormancy (Alderton *et al.*, 2017). However, the longevity and viability of *N. flexilis* seeds in dormant seedbanks is unknown and requires further investigation before this can be considered as an alternative source for reintroduction.

The exact location for reintroduction of *N. flexilis* seeds should be carefully considered in order to avoid disturbance, herbivory, competition or other sources of environmental stress that may prevent the plant from germinating and growing (Menges, 2008). Based upon previous knowledge, it is thought that *N. flexilis* should be introduced onto fine, silty sediments, in deep water (Wingfield *et al.*, 2004). This study has shown that *N. flexilis* naturally disperses seeds widely across lake basins. This strategy is often used by terrestrial plants to ensure that seeds reach a variety of different environmental conditions, increasing the likelihood that some will germinate and survive (MacArthur and Wilson, 1967; Kolar and Lodge, 2001). *N. flexilis* was also shown to grow in a variety of different micro-habitat conditions, dependent upon the environmental conditions and the community ecology of the lake in question. In some lakes, such as Loch Scaraidh, *N. flexilis* was found growing only at the deepest edge of the photic zone, whilst in others, such as Tangy Loch, it was found growing in waters as shallow as 0.08m. Whilst *N. flexilis* favoured silty sediments, it was also found growing on sand and rockier substrates. Whilst it is impractical to emulate the natural dispersal strategies of *N. flexilis* by introducing hundreds of seeds across entire lake basins, the results of this study imply that the introduction of multiple seeds in several different locations within a loch may be more successful than introductions at a single location. This is consistent with ecological theories that predict that the persistence time of an introduced population of any species increases with its initial population size (Robert *et al.*, 2007), however, of the 249 reintroduction attempts studied by Godefroid *et al.* (2011), 25% were based on fewer

than 50 individuals.

In England, both former *N. flexilis* sites (Esthwaite Water and Lake Windermere) are geographically distant and hydrologically isolated from sites at which *N. flexilis* is currently present. As such, if *N. flexilis* is to grow at these sites in the future, it must be either reintroduced or recovered from existing seedbanks. Following the measured recoveries of total phosphorus, chlorophyll-*a* and Secchi depth at Esthwaite Water since 2000, a project to find, germinate and reintroduce a *N. flexilis* seed from the existing seedbank has been launched (JNCC, 2012). To date, it has not been possible to grow germinated *N. flexilis* seeds on into plants, and reintroduction attempts have therefore been unsuccessful (Walmisley, pers. comms). This PhD provides some insight into the best approach to reintroductions, and also affords some longer-term context to the decline of *N. flexilis* at Esthwaite Water which may contra-indicate the reintroduction of the species at this site. Although *N. flexilis* was present at Esthwaite Water until severe nutrient enrichment occurred in the 1980s, its presence at the site has been sub-optimal since ~1915, when eutrophication was much less severe. Furthermore, the chemical recovery at Esthwaite Water has yet to be reflected in its ecology, suggesting that photosynthetic rates and consumption of carbon dioxide remain high. Until the alkalinity and concentrations of carbon dioxide in Esthwaite Water recover, it is unlikely to provide a favorable habitat for *N. flexilis.* It is therefore recommended that habitat restoration remains a priority over reintroduction until ecological recovery becomes evident.

7.5.4 Future Research

Whilst this PhD has advanced the understanding of the contemporary extent and decline of *N. flexilis* in the British Isles and provided insight into the "next steps" for protecting the species, it has also highlighted some areas in which further study would be beneficial. There remain some sites at which *N. flexilis* has been recorded in the past but the current status remains unknown (see Appendix 9). Additionally, there are some sites at which *N. flexilis* seeds have been detected in surface sediments, but the plant has not been recorded in contemporary surveys (e.g. Loch Flemington, Scotland (Bennion *et al.*, 2008), Upper

Lough Erne, Northern Ireland (Salgado, 2011)). The combination of the survey methodology presented in chapter 3 and the development of eDNA techniques for the detection of *N. flexilis* in lake water and sediment samples mean that the chances of detecting *N. flexilis* at sites where it is present are much improved. The unsurveyed sites represent an opportunity to test these new methods whilst improving our understanding of the contemporary distribution of *N. flexilis* in the British Isles.

The PhD thesis of Wingfield (2002) and the work presented in chapter 4 provide a good understanding of the chemical and physical conditions in which *N. flexilis* thrives, but it has not yet been possible to study the influence of seasonal fluctuations on the plant. Seasonality could play an important role in determining whether the plant is able to grow in any given location. For example, *Najas marina* seeds require a period of cold stratification followed by spring warming to awake from dormancy, preventing it from growing in northern lakes where spring warming occurs too late in the season for germination to occur (Handley and Davy, 2005). Investigation of the influence of seasonal trends on *N. flexilis* growth could help to further explain the changing distribution of the plant. Additionally, this study has highlighted that nitrogen could have an important limiting role on *N. flexilis* growth. This echoes a growing body of evidence that some aquatic organisms and lake ecosystems are either nitrogen limited or co-limited (e.g. Moss *et al.*, 1994; Maberly *et al.*, 2002; Jones *et al.*, 2004), and there is now concern that, in such instances, lake eutrophication could be spurred by atmospheric deposition of nitrogen (Bergström and Jansson, 2006). In light of this, it is recommended that further investigations into the role of nitrogen on *N. flexilis* growth be conducted.

Although *N. flexilis* seeds are well preserved in lake sediments, paleoecological studies of the plant remain few and far between. The research presented in chapter 6 demonstrates the value of paleoecology in determining the causes and timing of *N. flexilis* decline and loss at selected sites. However, as demonstrated in chapter 4, the range of communities within which *N. flexilis* can grow is broad, and the sites studied in this PhD do not cover them all. Furthermore, all sites at which paleoecological studies have been conducted assess long-term changes resulting from local anthropogenic forcing (e.g. Esthwaite

Water in this thesis) or relate to past geological epochs (e.g. Galka *et al.*, 2012). In catchments isolated from pollution, paleoecology offers the opportunity to study the impact that contemporary climate change is having on *N. flexilis.* Climate change was hypothesised as a threat to *N. flexilis* by Wingfield (2002) but has not yet been investigated, and paleoecological studies from more sites could help to elucidate the nature of this threat.

In terms of restoring lost populations of *N. flexilis,* this study suggests that habitat restoration be prioritised. In cases of high phosphorus loadings, this can often be achieved over long timescales using catchment management schemes. Where such schemes are in place, continued long-term monitoring is recommended, and, where successful, could provide insight into the mechanisms by which *N. flexilis* colonises new sites. In order to facilitate the growth of *N. flexilis* at such sites, the spread of *Elodea* spp. needs to be managed, and research into methods for removing the species without damaging *N. flexilis* populations should be explored. In sites where environmental conditions have been restored and are suitable for *N. flexilis* growth, the restoration of the species will be reliant on the presence of viable seeds. Although chapter 5 shows that *N. flexilis* seeds are often abundant in sediments, the viability of these seeds - particularly in lakes where *N. flexilis* has not grown for some time - is not known. Studies investigating the longevity and dormancy of seeds in the seedbank could greatly assist future attempts to reintroduce *N. flexilis* as sites recover from environmental degradation.

7.6 Implications for paleoecology and conservation science

This PhD research has extended existing knowledge on the history and ecology of *N. flexilis* in the UK, providing valuable insights into current threats to the species and potential strategies for mitigating future losses. In doing this, it has addressed a common problem in conservation science, namely the lack of data to drive evidence-based conservation practices. Paleoecology is often used to extend contemporary environmental records into the past, largely in application to community-scale ecology (Graumlich *et al.*, 2005). Recently, the long-term perspective on ecosystem change offered by

paleoecology has been used to advise on a range of lake management issues, including the necessity of nutrient reduction measures, sediment removal to expose macrophyte seed banks, fish management, and fish farm licensing (Sayer *et al.*, 2012). However, despite strong arguments supporting the use of paleoecology to extend species-level records of biodiversity into the past, such techniques have generally been considered too qualitative and imprecise to be useful in practical conservation practices (Willis and Birks, 2006). Because of this, *"how can paleoecological data be used to inform ecosystem restoration, species recovery and reintroductions?"* has been identified as one of the fifty most pressing questions in paleoecology today (Seddon *et al.*, 2014). By quantitatively assessing the probable seed numbers found in the surface sediments of lakes with "abundant", "occasional", and "extinct" contemporary populations of *N. flexilis,* this study has been able to apply a semi-quantitative approach to the interpretation of down-core changes in numbers of *N. flexilis* seeds during paleoecological studies. At Esthwaite Water, this technique led to the identification of a previously unknown period of *N. flexilis* abundance at the site prior to the first historical botanical record, and highlighted the full extent of the impacts of nutrient enrichment during the first half of the twentieth century. At Loch of Craiglush, the same approach suggested that, despite several historical botanical records of *N. flexilis* made at the site, it is unlikely that the loch has supported *N. flexilis* in abundance during the last ~200 years, and that the neighbouring Loch of Butterstone, which has been more severely impacted by nutrient enrichment, was likely a more suitable habitat for the species. Thus, paleoecology can be used to identify former environmental conditions associated with *N. flexilis*, and this information can now be used to set targets for habitat restoration.

Whilst the results of the paleoecological work provide long-term context to *N. flexilis* loss, the specific conclusions drawn are relevant only to the sites studied directly. Since paleoecological work can be expensive and time-consuming, such studies are rarely applied across a wide spatial scale. This means that paleoecological studies usually cover only a small subsection of the total range of environmental variability found. For example, the macrofossil reconstruction of Loch of Craiglush, when taken alone, implies that *N. flexilis* is associated with acid-tolerant flora such as *Juncus bulbosus.* Whilst this

is true for this site, it is clearly not the only plant sociology situation in which *N. flexilis* can be found naturally. This has implications for the interpretation of paleoecological results and subsequent management actions; at Loch of Craiglush, creation of conditions that support a more acid-tolerant flora than currently found would not likely restore a healthy population of *N. flexilis,* which has been shown to grow more abundantly at higher pH provided other conditions are favourable. By making use of contemporary monitoring data across a wide range of different *N. flexilis* sites, this study demonstrates that the plant is able to occupy lakes with different environmental conditions, and that changes in these environmental conditions may alter the ability of *N. flexilis* to compete with other plants. Such insight into potential competition on a micro-habitat scale would not be possible using paleoecology alone. Furthermore, unless multiple sediment cores are taken in a single basin (e.g. Madgwick *et al.*, 2011), paleoecology is rarely able to provide an insight into processes occurring on the micro-habitat scale. In the case of *N. flexilis,* contemporary ecological data revealed that, in lakes with higher alkalinities, the species can only survive where other plants are not competing for carbon dioxide. This helps to explain the disappearance of the plant with eutrophication observed in the paleoecological record, despite its tolerance for low light levels and fluid sediments. For this reason, it is commonly recommended that evidence for specific biological responses to environmental management be monitored using direct observational data (Battarbee *et al.*, 2005). However, this PhD clearly demonstrates that, in combination, contemporary ecological studies and paleoecology can provide a more substantial evidence base for the conservation of rare species than can be obtained from either discipline in isolation.

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Appendices

Appendix 1: Summary of *N. flexilis* records made in the British Isles, 1850 – present

Region	County	Site name	Grid reference	Date of last survey	Date *N. flexilis* first recorded	Date *N. flexilis* last recorded	Source
Sites where *N. flexlis* recorded since 2007							
Mainland Scotland	Argyll	Loch Nan Gad	NR787575	2009	1971	2009	National Biodiversity Network and SNH
		Tangy Loch	NR695280	2014	1973	2014	Direct observation
	Perthshire	White Loch	NO1642	2010	1877	2010	SNH
Inner Isles	Coll	Loch an t-Sagairt	NM250610	2009	1989	2009	SNH
		Loch Ballyhaugh	NM176581	2016	1987	2016	Direct observation
	Colonsay	Loch Fada	NR383955	2009	1902	2009	SNH
		Glenastle Loch	NR300447	2013	1950	2013	Direct observation
	Islay	Loch Gorm	NR230655	2008	1994	2008	SNH
		Loch Lossitt	NR408652	2010	1993	2010	SNH
		Loch Skerrols	NR3463	2010	1949	2010	SNH
		Lower Glenastle Loch	NR294450	2008	1949	2008	SNH
Western Isles (Outer Hebrides)	North Uist	Loch Eaval	NF7271	2010	1977	2010	SNH
		Loch Runavat	NF730693	2010	1974	2010	SNH
		Loch Scaraidh	NF718705	2016	1994	2016	Direct observation

	South Uist	Loch a'Mhadaidh	NF762353	2016	1994	2016	Direct observation
		Loch Aiseabhat	NF757153	2008	2008	2008	SNH
		Loch an Eilean (Drimsdale)	NF762372	2016	1984	2016	Direct observation
		Loch Cuilc	NF772369	2016	1994	2016	Direct observation
		Loch Druidibeg	NF7937	2016	1987	2016	Direct observation
		Loch an Eilean (S. Boisdale)	NF748169	2010	1983	2010	SNH
		Loch Gearraidh Mhic Iain	NF763360	2016	1994	2016	Direct observation
		Loch na Cuithe Moire	NF738234	2016	1983	2016	Direct observation
		Loch nan Capull	NF754161	2010	2010	2010	SNH
		Loch nan Cnamh	NF762355	2016	1994	2016	Direct observation
		Mid Loch Ollay	NF748319	2016	1987	2016	Direct observation
		Schoolhouse Loch	NF763364	2016	1983	2016	Direct observation
Ireland*	Donegal	Lough Akibbon	C068183	2010	1977	2010	C. Roden and A. O'Conner (pers. comms.)
		Lough an tSeisigh	C040360	2010	1981	2010	C. Roden and A. O'Conner (pers. comms.)
		Lough Anure	B8235315149	2009	2009	2009	C. Roden and A. O'Conner (pers. comms.)
		Lough Fern	C1808922480	2010	2009	2010	C. Roden and A. O'Conner (pers. comms.)
		Lough Kiltooris	G6896	2010	1989	2010	C. Roden and A. O'Conner (pers. comms.)
		Lough Kindrum	C14	2016	1916	2016	C. Roden and A. O'Conner (pers. comms.)

	Galway	Lettershask East	L6342	2014	1975	2014	C. Roden and A. O'Conner (pers. comms.)
		Loch na Creibhinne	L989211	2016	2005	2016	C. Roden and A. O'Conner (pers. comms.)
		Lough Anaserd	L610440	2009	1936	2009	C. Roden and A. O'Conner (pers. comms.)
		Lough Ballynakill	L 64766 58149	2016	2003	2016	C. Roden and A. O'Conner (pers. comms.)
		Lough Bofin	M0253544272	2010	2007	2010	C. Roden and A. O'Conner (pers. comms.)
		Lough Illauntrasna	L7557	2014	1977	2007	C. Roden and A. O'Conner (pers. comms.)
		Lough Maumeen	L683475	2000	1977	1977	C. Roden and A. O'Conner (pers. comms.)
		Loughauneala	L930233	2010	2005	2010	C. Roden and A. O'Conner (pers. comms.)
	Kerry	Long Range, Killarney	V9383	2016	1994	2016	C. Roden and A. O'Conner (pers. comms.)
		Lough Acoose	V78	2011	1899	2011	C. Roden and A. O'Conner (pers. comms.)
		Lough Caragh	V7088	2016	1877	2016	C. Roden and A. O'Conner (pers. comms.)
		Lough Leane	V98	2013	1886	2013	C. Roden and A. O'Conner (pers. comms.)
		Upper Lake	V88	2014	1906	2014	C. Roden and A. O'Conner (pers. comms.)
	Mayo	Lough Moher	L9775276494	2008	2008	2008	C. Roden and A. O'Conner (pers. comms.)
Sites where *N. flexilis* not present in last survey							
Mainland Scotland	Central Region	Lake of Menteith	NS577002	2009	1994	2004	SNH
	Dumfries and Galloway	Loch Kindar	NX967642	2017	1959	2002	Wingfield, Evers and Murphy (2002) and ECUS (2017, pers. comms.)
	Fife	Lindores Loch	NO2616	2000	1874	1878	NBN and SNH

	Perthshire	Loch Clunie	NO116444	2016	1870	2004	SNH (2004) and direct observation (2016)
		Loch Marlee (Drumellie)	NO1444	2016	1877	2007	Benthic Solutions (2007) and direct observation (2016)
		Loch Monzieviard	NN8423	2000	1880	1884	Natural History Museum London, herbarium specimen collected by R. Kidston
		Loch of Butterstone	NO0644	2016	1986	2004	SNH (2004) and direct observation (2016)
		Loch of Craiglush	NO044446	2016	1967	2004	SNH (2004) and direct observation (2016)
		Loch of Lowes	NO055443	2016	1879	2007	Benthic Solutions (2007) and direct observation (2016)
		Monk Myre	NO2042	1999	1879	1883	National Biodiversity Network and SNH.
	Westerness	Loch an Eilean	NF766377	1999	1983	1994	Chris Preston, pers. comms.
Western Isles (Outer Hebrides)	North Uist	Loch Grogary	NF712715	2016	1942	2010	SNH
	South Uist	Loch ? (no name)	NF766369	2016	1994	2000	SNH
		Loch Bun an Ligidh	NF769371	2016	1994	2000	SNH
		West Loch Ollay	NF737327	2016	1940	2001	SNH
England	Cumbria	Esthwaite Water	SD360965	2014	1914	1982	SNH
		Lake Windermere	SD390956	2014	1977	1977	SNH
Ireland	Donegal	Lough Ibby	B72	2002	1919	1955	C. Roden and A. O'Conner (pers. comms.)
	Galway	Lettershask West	L6243	1988	1975	1975	C. Roden and A. O'Conner (pers. comms.)
		Lough Anillaunlughy	L64	2014	1973	2004	C. Roden and A. O'Conner (pers. comms.)

		Lough Bollard	L64	2004	1973	1973	C. Roden and A. O'Conner (pers. comms.)
		Lough Corrib	L9851	2014	1986	1986	C. Roden and A. O'Conner (pers. comms.)
		Lough Kylemore or Pollacappul	L653406	2016	2005	2016	C. Roden and A. O'Conner (pers. comms.)
		Lough Nafeakle	L8889225105	2007	2007	2007	C. Roden and A. O'Conner (pers. comms.)
		Lough Namanawaun	L6740	2003	1851	1975	C. Roden and A. O'Conner (pers. comms.)
		Truska Lough	L6740	2004	1975	1975	C. Roden and A. O'Conner (pers. comms.)
		Cloonee Lough Middle	V86	2014	1957	1957	C. Roden and A. O'Conner (pers. comms.)
	Kerry	Lough Adoolig	V645740	2014	1981	1995	C. Roden and A. O'Conner (pers. comms.)
		Muckross Lake	V98	2014	1899	1976	C. Roden and A. O'Conner (pers. comms.)
		Lough Glenade	G828461	2014	1977	1978	C. Roden and A. O'Conner (pers. comms.)
	Leitrim	Fin Lough	L8466	2014	1937	1937	C. Roden and A. O'Conner (pers. comms.)
	Mayo	Keel Lough	F650055	2014	1999	2004	C. Roden and A. O'Conner (pers. comms.)
Sites where current *N. flexilis* status unknown							
Mainland Scotland	Perthshire	River Tay, Aberfeldy	NN84	1929	1929	1929	Natural History Museum London herbarium specimen, collected by R. Meinertzhagen.
		Fingask Loch	NO165431	2006	1877	2006	N. Stewart macrophytes course 2006 (pers. comms.)

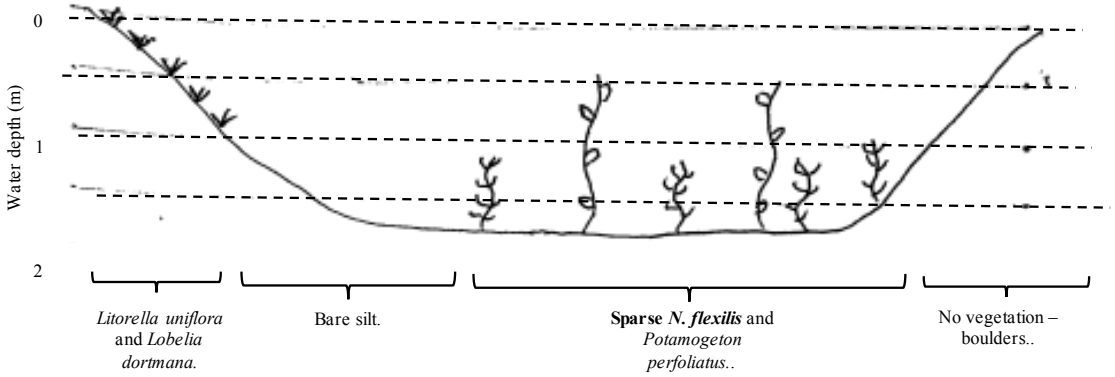
	Ross, Skye and Lochaber	Loch a' Bhada Daraich	NM6994	2000	1977	2000	SNH
Inner Isles	Mull	Loch Poit na h-I	NM314227	1999	1994	1999	National Biodiversity Network
Western Isles (Outer Hebrides)	Benbecula	Benbecula aerodrome	NF7956	1950	1950	1950	SNH
		Loch Dun Mhurchaidh	NF7954	1940	1940	1940	SNH
	North Uist	Loch Leodasay	NF8063	1982	1982	1982	SNH
		Loch nam Margalan	NF7270	1942	1942	1942	SNH
	South Uist	Loch a'Mhuilinn	NF7433	1951	1947	1951	SNH
		Loch Altabrug	NF745340	2000	1983	2000	SNH
		Loch Ceann a Bhaigh	NF7630	1940	1940	1940	SNH
		Loch Eilean a'Ghille-Ruaidh	NF769366	1999	1983	1999	SNH
		Loch nam Faoileann	NF752210	2000	1995	2000	SNH
?	?	Loch na Paice Dubh	?	1908	1908	1908	Natural History Museum London herbarium specimen, collected by M. Mcneill
England	Manchester	"Manchester pond"	?	1883	1983	1883	SNH
Ireland	?	Ainlough	?	1885	1885	1885	C. Roden and A. O'Conner (pers. comms.)
	Donegal	Clooney Lough	G79	2002	1939	2002	C. Roden and A. O'Conner (pers. comms.)
		Loch an Phoirt	C0034	2006	1989	2006	C. Roden and A. O'Conner (pers. comms.)
		Lough Mullaghderg	B71	2002	1919	2002	C. Roden and A. O'Conner (pers. comms.)

		Lough Nagreaney	C1441	2002	1990	2002	C. Roden and A. O'Conner (pers. comms.)
		Lough Seskinmore	G700960	2004	1981	2004	C. Roden and A. O'Conner (pers. comms.)
		Lough Shannagh	C2045	2002	1989	2002	C. Roden and A. O'Conner (pers. comms.)
	Galway	Loch an Chaolaigh	L804307	2004	1996	2004	C. Roden and A. O'Conner (pers. comms.)
		Loch Nageeron	L73	2004	1974	2004	C. Roden and A. O'Conner (pers. comms.)
		Lough Barnahallia	L593556	2004	1975	2004	C. Roden and A. O'Conner (pers. comms.)
		Lough Cregduff	L73	2004	1852	2004	C. Roden and A. O'Conner (pers. comms.)
		Lough Derrywaking	L6748	2004	1989	2004	C. Roden and A. O'Conner (pers. comms.)
		Lough Emlaghnacourty	L654459	2004	1999	2004	C. Roden and A. O'Conner (pers. comms.)
		Lough Lehanagh	L784315	2004	1990	2004	C. Roden and A. O'Conner (pers. comms.)
		Lough Keeraun	L85	2004	1975	2004	C. Roden and A. O'Conner (pers. comms.)
		Lough Nalawney	L690415	1995	1977	1995	C. Roden and A. O'Conner (pers. comms.)
		Lough Natawnymore	L92	2004	1990	2004	C. Roden and A. O'Conner (pers. comms.)
		Lough Rusheenduff	L66	2004	1935	2004	C. Roden and A. O'Conner (pers. comms.)
		Lough Truskan	L808305	2004	1990	2004	C. Roden and A. O'Conner (pers. comms.)
		Lough Tully	L692616	2004	1978	2004	C. Roden and A. O'Conner (pers. comms.)

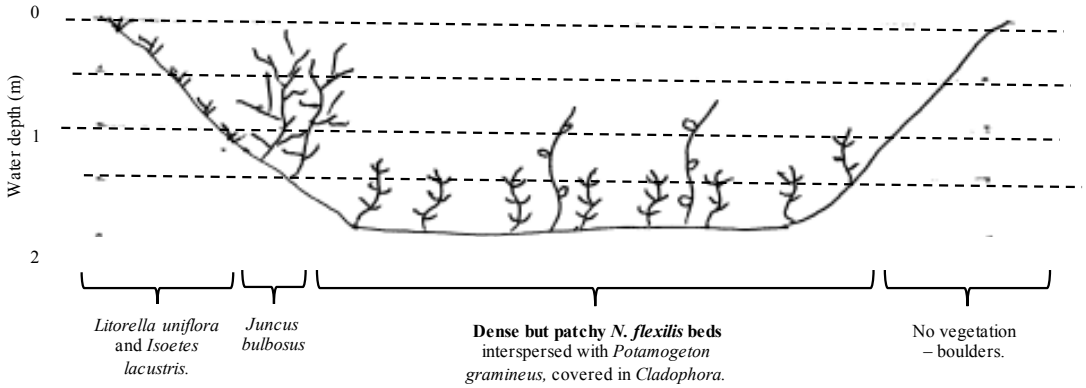
		Roundstone	L73	1907	1833	1907	C. Roden and A. O'Conner (pers. comms.)
		Roundstone	L73	1896		1896	C. Roden and A. O'Conner (pers. comms.)
		Seanchrois	L632430	2005	1999	2005	C. Roden and A. O'Conner (pers. comms.)
	Mayo	Lough Dahybaun	G01	2004	1977	2004	C. Roden and A. O'Conner (pers. comms.)
		Lough Nageltia	M114785	2004	2004	2004	C. Roden and A. O'Conner (pers. comms.)
		Lough Nahaltora	L793741	2004	2000	2004	C. Roden and A. O'Conner (pers. comms.)
		Lough Dhu	?	1896	1896	1896	C. Roden and A. O'Conner (pers. comms.)

Appendix 2: Loch of Glenastle SCUBA transect diagrams

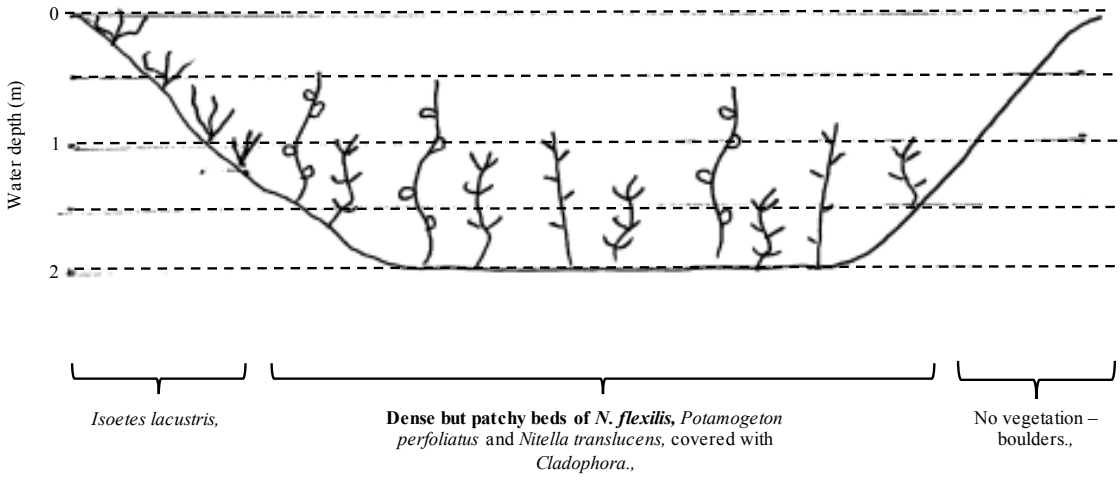
Transect 1 (westernmost transect)



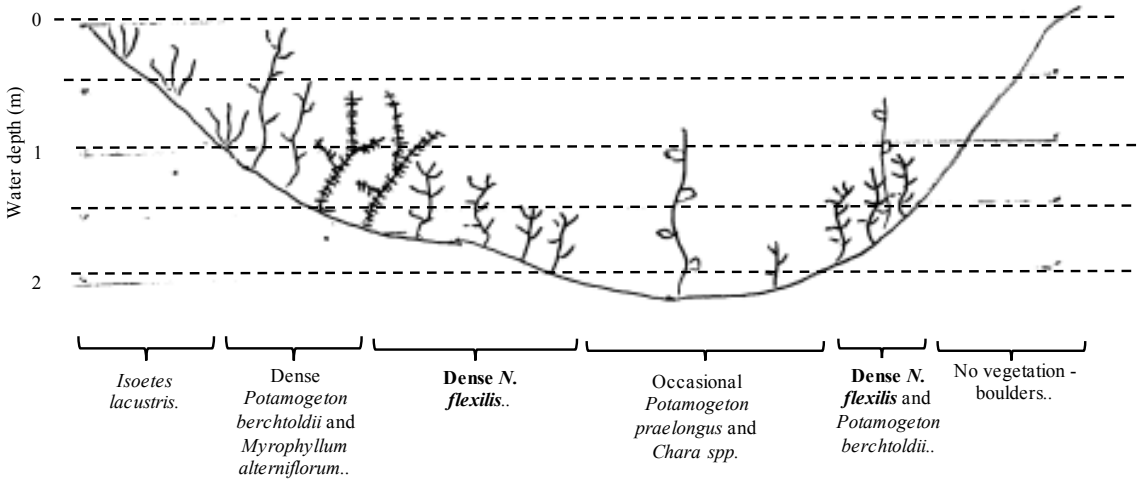
Transect 2



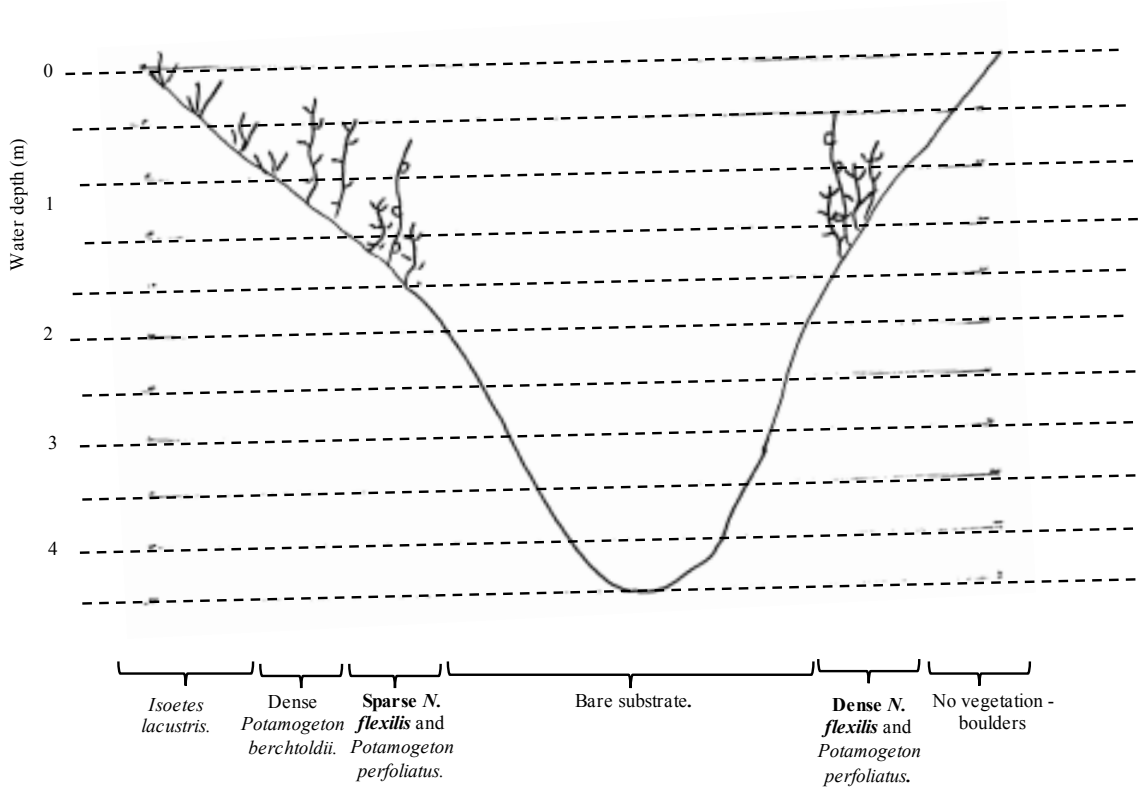
Transect 3



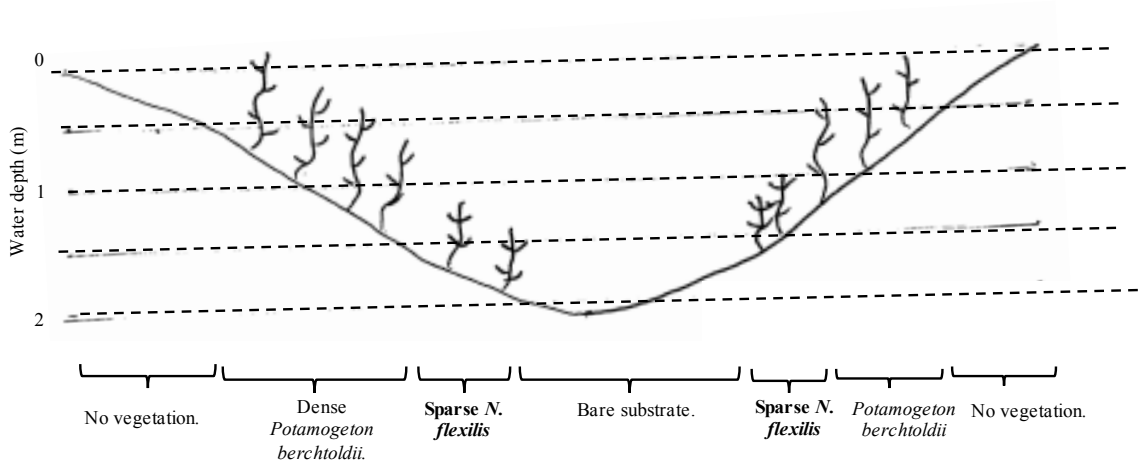
Transect 4



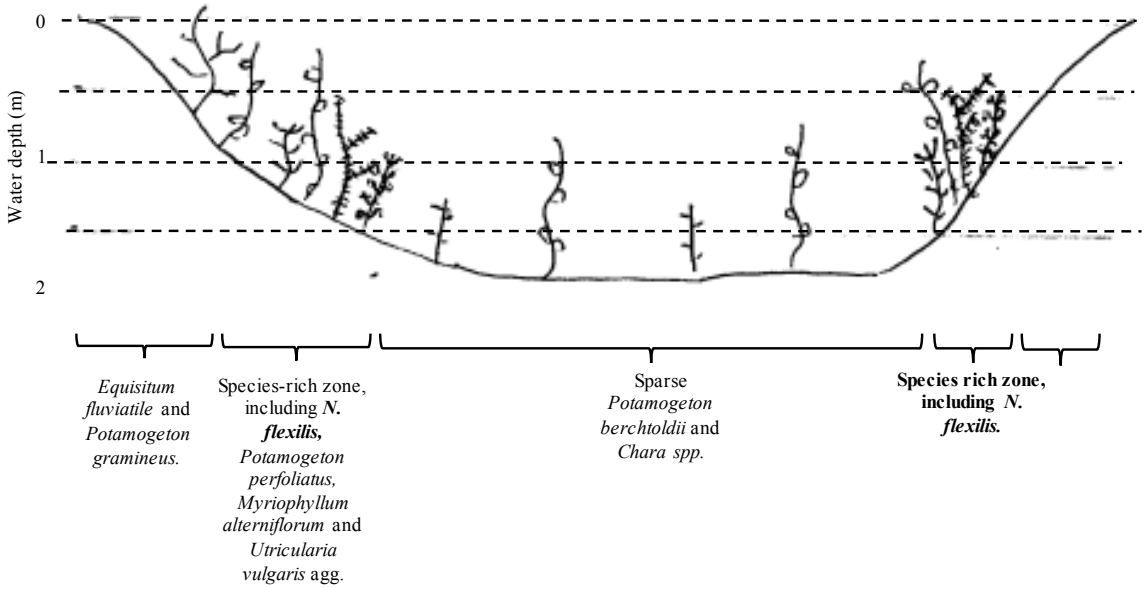
Transect 5



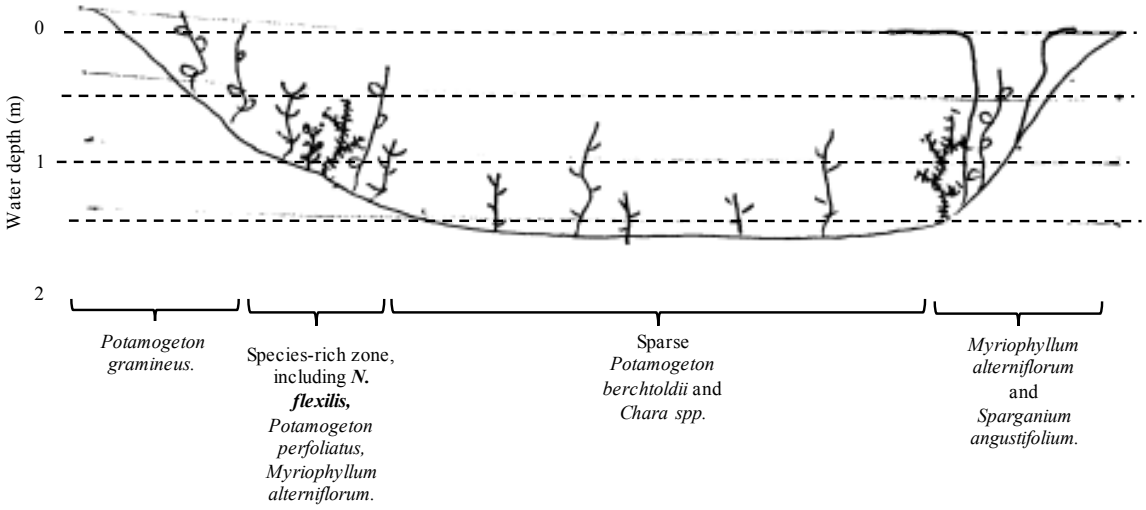
Transect 6



Transect 7



Transect 8 (easternmost transect)



Appendix 3: Water chemistry monitoring data for SCM sites where *N. flexilis* present and absent

Site	Survey year	N. flexilis %cover	Alkalinity as CaCO3 (mg/l)	Conductivity (µS/cm)	DO (mg/l)	LEC	NO3 (mg/l)	pH	Salinity (ppm)	TDS (ppm)	Total P (mg/l)
***N. flexilis* present**											
Loch a' Mhadaidh	2016	3	No data	167	No data	1.77	No data	7.38	No data	No data	No data
Loch an Eilean	2004	30	No data	235	No data	1.96	No data	7.50	No data	No data	No data
	2010	22	No data	235	No data	1.96	No data	7.50	No data	No data	No data
	2016	19	No data	235	9.35	0.85	No data	7.20	0.04	No data	No data
Loch an T'Sagairt	2009	1	No data	163	No data	3.05	No data	6.90	No data	No data	No data
Loch Ballyhaugh	2004	75	No data	284	9.17	2.85	No data	7.40	0.11	114	No data
	2009	28	No data	229	9.17	2.85	No data	6.75	0.11	114	No data
	2016	34	No data	229	9.17	2.85	No data	6.75	0.11	114	No data

Loch Cuilc	2016	16	No data	100	No data	1.13	No data	6.90	0.08	No data	No data
Loch Druidibeg	2004	2	5.40	130	No data	No data	0.05	6.64	No data	No data	0.01
	2010	32	5.67	116	No data	No data	0.08	6.81	No data	No data	0.01
	2016	4	3.79	131	10.30	No data	0.15	6.55	0.09	98	0.01
Loch Fada	2004	33	No data	293	No data	3.19	No data	6.90	No data	No data	No data
	2009	31	No data	293	No data	3.19	No data	6.90	No data	No data	No data
Loch Gearraidh Mhic Iain	2016	15	No data	226	No data	1.96	No data	7.45	No data	No data	No data
Loch Glenastle	2013	46	12.12	127	No data	No data	0.14	7.25	No data	No data	0.01
Loch Grogary	2004	4	38.85	241	No data	No data	0.08	7.55	No data	No data	0.03
	2010	17	64.45	No data	No data	No data	0.07	8.06	No data	No data	0.03
Loch na Cuithe Moire	2004	32	19.15	183	No data	4.07	0.02	7.30	No data	No data	0.00
	2010	22	19.15	183	No data	4.07	0.02	7.30	No data	No data	0.00
	2016	28	19.15	257	3.03	1.70	No data	7.42	No data	167	No data
Loch Nam Cnamh	2016	46	32.40	230	No data	1.65	No data	7.48	No data	No data	0.00
Loch Phuirt Midh	2016	26	No data	198	10.73	0.85	No data	6.94	0.09	99	No data

Loch Scaraidh	2016	1	No data	329	9.20	1.70	No data	9.92	0.01	213	No data
Loch Tangy	2013	100	23.27	129	No data	No data	0.15	7.39	No data	No data	0.02
Mid Loch Ollay	2004	32	No data	199	6.46	1.89	No data	8.23	0.06	128	No data
	2010	41	No data	199	6.46	1.89	No data	8.23	0.06	128	No data
	2016	9	No data	199	6.46	1.89	No data	8.23	0.06	128	No data
Schoolhouse Loch	2016	28	No data	369	6.19	1.31	No data	6.94	0.04	74	No data
***N. flexilis* absent**											
Lake of Menteith	2009	0	9.70	77	No data	0.62	0.01	7.00	No data	No data	0.01
Loch ?	2016	0	24.51	405	No data	1.91	No data	7.50	No data	No data	0.02
Loch an T'Sagairt	2004	0	No data	163	No data	3.05	No data	6.90	No data	No data	No data
Loch Bun an Ligidh	2016	0	8.61	97	No data	1.13	0.01	6.80	No data	No data	0.01
Loch Clunie	2004	0	No data	198	No data	1.33	0.02	8.00	No data	No data	No data
	2010	0	No data	198	No data	1.33	0.02	8.00	No data	No data	No data
	2016	0	No data	198	No data	0.94	0.02	8.00	No data	No data	No data
Loch Grogary	2016	0	No data	265	No data	0.94	0.01	7.70	No data	No data	No data

Loch Marlee	2010	0	No data	214	No data	1.46	0.02	9.00	No data	No data	No data
	2016	0	No data	214	No data	1.46	0.02	9.00	No data	No data	No data
Loch of Butterstone	2004	0	No data	139	No data	4.06	0.01	8.30	No data	No data	No data
	2010	0	No data	139	No data	No data	0.01	8.30	No data	No data	No data
	2013	0	No data	263	No data	1.42	0.00	8.00	No data	No data	No data
	2016	0	No data	139	No data	1.70	0.01	8.30	No data	No data	No data
Loch of Craiglush	2016	0	16.00	127	No data	2.98	No data	7.50	No data	No data	0.01
Loch of Lowes	2010	0	23.45	126	No data	1.76	No data	7.50	No data	No data	0.03
	2016	0	23.45	126	No data	1.76	No data	7.50	No data	No data	0.03
Loch Scaraidh	2010	0	102.32	416	No data	No data	0.07	8.26	No data	No data	0.02
West Loch Ollay	2004	0	No data	No data	No data	No data	No data	No data	No data	No data	No data
	2010	0	No data	No data	No data	No data	No data	No data	No data	No data	No data
	2016	0	No data	No data	No data	No data	No data	No data	No data	No data	No data

Appendix 4: List of macrophyte species found at current and former *N. flexilis* sites during SCMs

Species name (as recorded)	Abbreviation
Najas flexilis	NFlex
Agrostis stolonifera	AStol
Alismataceae sp.	Alis
Alnus glutinosa	AGlut
Apium inundatum	AInund
Baldellia ranunculoides	BRan
Blindia acuta	BAcu
Brachythecium rutabulum	BRut
Calliergon cuspidatum	CCusp
Callitriche sp	Call
Callitriche brutia	CBrut
Callitriche brutia var. hamulata	CBruHam
Callitriche hamulata	CHam
Calitriche hermaphroditica	CHerm
Callitriche platycarpa	CPlat
Caltha palustris	CPal
Carex rostrata	CRost
Carex lepidocarpa	CLep
Carex nigra	CNig
Carex panicea	CPan
Chara aspera	ChAsp
Chara contraria	ChCont
Chara curta	ChCurt
Chara hispida	ChHisp
Chara rudis	ChRud
Chara sp	Chara
Chara virgata	ChVirg
Cladophora sp	Clad
Drepanocladus sp.	Drep
Eleocharis multicaulis	EMult
Elatine hexandra	EHex
Elatine hydropiper	EHydro
Elatine sp	Elat
Eleocharis acicularis	EAcic
Eleocharis multicaulis	EMCaul

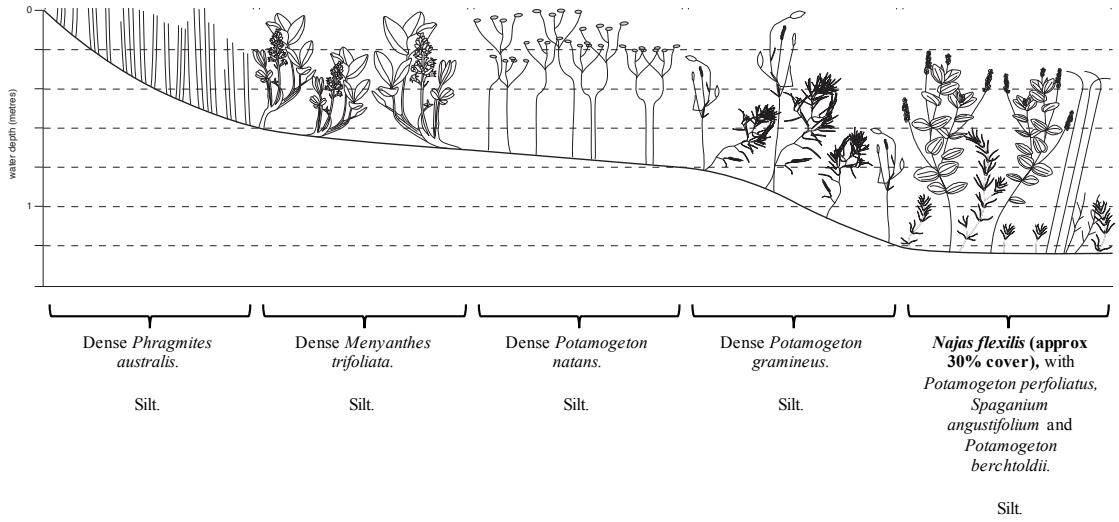
Eleocharis palustris	EPal
Eleocharis sp	Eleoc
Eleogiton fluitans	EFluit
Elodea canadensis	ECan
Elodea nuttallii	ENutt
Equisetum fluviatile	EqFlu
Eriophorum angustifolium	ErAng
Fontinalis antipyretica	FAnt
Galium palustre	GPal
Glyceria fluitans	GlyFlu
Glyceria maxima	GlyMax
Hydrocotyle vulgaris	HVulg
Iris psuedacorus	IPsued
Isoetes sp.	Isoete
Isoetes echinospera	IEch
Isoetes lacustris	ILac
Juncus articulatus	JArt
Juncus bulbosis	JBulb
Lemna minor	Lmin
Lemna triscula	LTrisc
Littorella uniflora	LittUni
Lobelia dortmana	LDort
Lythrum portula	LytPort
Mentha aquatica	MAquat
Menyanthes trifoliata	MTrif
Molinia caerulea	MCaer
Moss sp	Moss
Myosotis laxa	MLaxa
Myrica gale	MGale
Myriophyllum alterniflorum	Malt
Myriophyllum sp	Myrio
Myriophyllum spicatum	MSpic
Nitella conferacaea	NConf
Nitella flexilis agg	NitFlex
Nitella sp	Nitel
Nitella translucens	NTrans
Nostoc	Nostoc
Nuphar lutea	NLut
Nuphar pumila	NPum
Nuphar x spenneriana	NxSpen

Nymphaea alba	NAlba
Persecaria amphibia	PAmph
Phalaris arundinacea	PhArund
Phragmites australis	PhAus
Pilularia globulifera	PGlob
Potamogeton berchtoldii	PBerch
Potamogeton crispus	PCrisp
Potamogeton filiformis	PFili
Potamogeton friesii	Pfries
Potamogeton gramineus	PGram
Potamogeton lucens	PLuc
Potamogeton natans	PNat
Potamogeton obtusifolius	PObt
Potamogeton pectinatus	PPect
Potamogeton perfoliatus	PPerf
Potamogeton polygonifolius	PPoly
Potamogeton praelongus	PPrae
Potamogeton pusillus	PPus
Potamogeton rutilis	PRut
Potamogeton sp	Pot
Potamogeton x nitens	PxNit
Potentilla anserina	PAns
Potentilla palustrus	PPal
Ranunculus aquatilis agg.	RAquat
Ranunculus flammula	RFlam
Ranunculus repens	RRep
Rorippa nasturtium-aquaticum agg.	RNast
Salix cinerea	SCin
Schoenoplectus lacustris	SchLac
Scorpidium scorpiodes	SScorp
Solanum dulcamara	SDulc
Sparganium angustifolium	SpAng
Sparganium erectum	SpErect
Sparganium natans	SpNat
Sphagnum sp	Sphag
Sponge	Sponge
Subularia aquatica	SubAquat
Triglochin maritima	TMarit
Utricularia intermedia agg	UInt

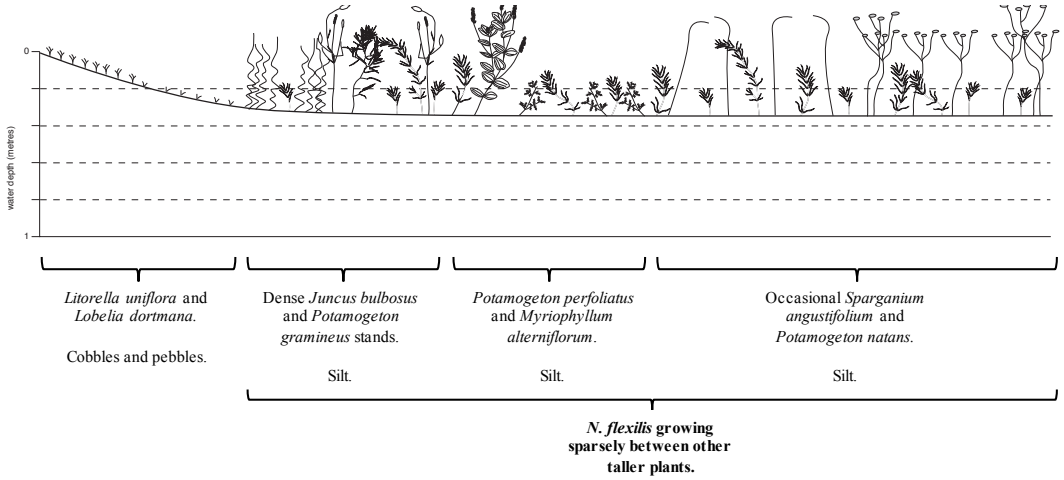
Utricularia minor	Umin
Utricularia sp	Utric
Utricularia stygia	UStyg
Utricularia vulgaris agg	UVulg
Zannichellia palustris	ZPal

Appendix 5: SCM snorkel transect diagrams

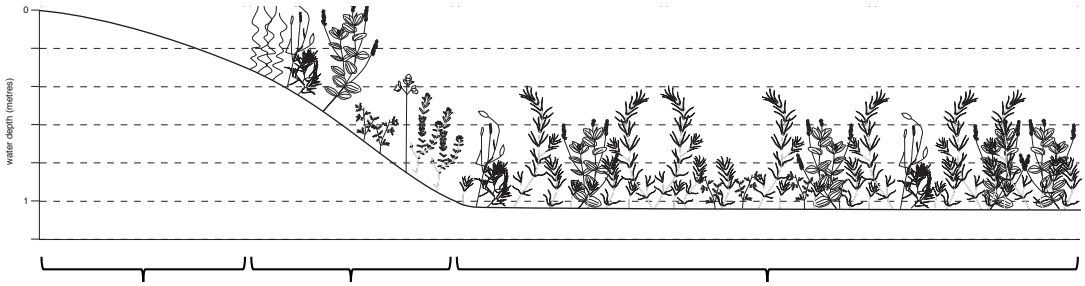
Loch Ballyhaugh



Loch Cuilc 1 and 2



Loch a'Mhadaidh



No vegetation.

Boulders.

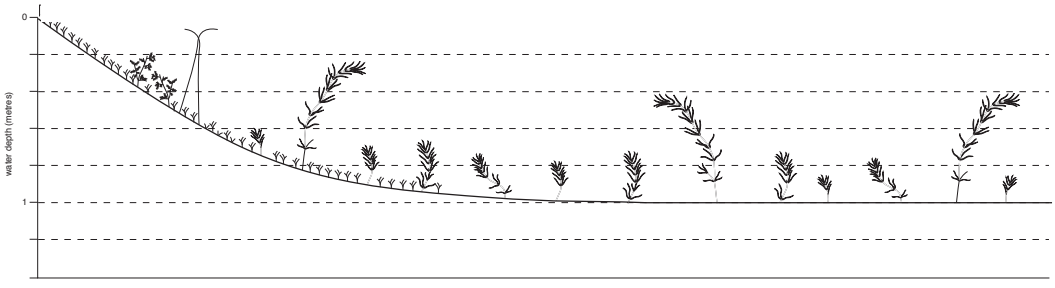
Very species rich zone, including *Juncus bulbosus, Potamogeton gramineus, Potamogeton perfoliatus, Myriophyllum alterniflorum, Utricularia vulgaris* agg., and *Callitriche bruttia*.

Silt..

Dense, monospecific stands of *N. flexilis* (up to 100% cover), alternating with stands of *Callitriche hermaphroditica,* interspersed with occasional *Potamogeton perfoliatus* and *Potamogeton gramineus.*

Silt..

Loch Bun an Ligidh



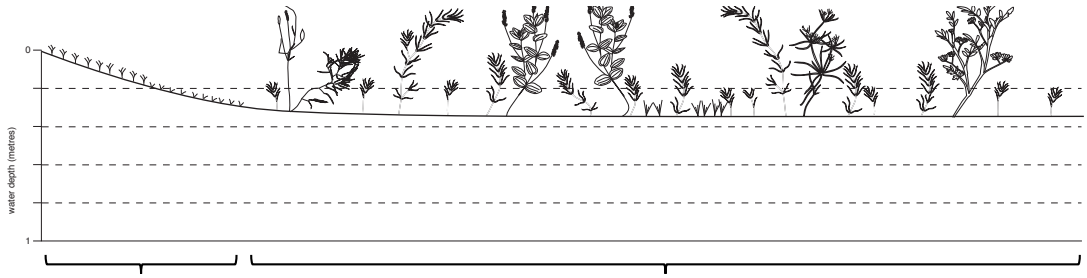
Dense *Litorella uniflora,* with occasional *Myriophyllum alterniflorum, Sparganium angustifolium* and *Lobelia dortmana.*

Sand and silt.

Occasional *N. flexilis* throughout (maximum 5% cover).

Silt.

Loch na Cuithe Moire 1



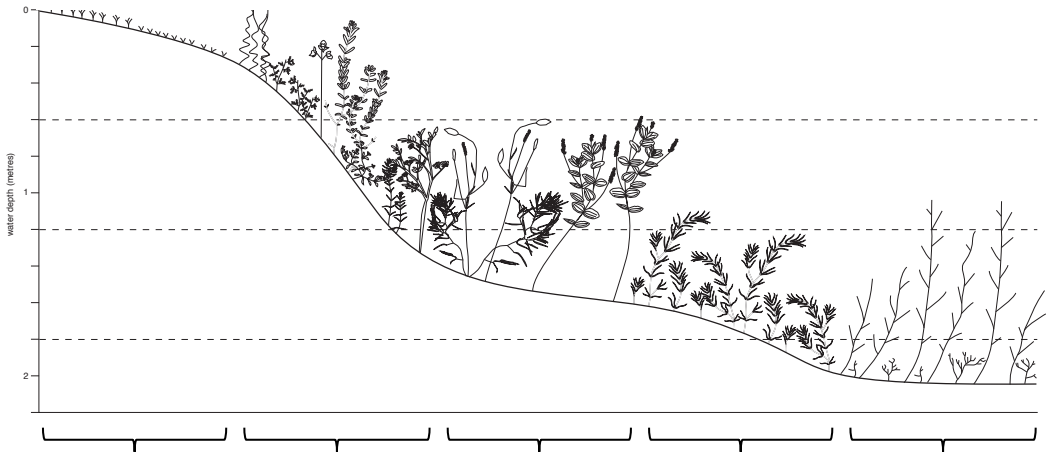
Litorella uniflora and *Lobelia dortmana.*

Sand and silt.

Occasional *N. flexilis* (max 10% cover), scattered amongst diverse range of other species including *Potamogeton gramineus, Potamogeton perfoliatus, Isoetes lacustris, Nitella translucens* and *Apium inundatem.*

Silt.

Loch na Cuithe Moire 2



Litorella uniflora and *Lobelia dortmana.*

Sand.

Very species rich zone, including *Juncus bulbosus, Myriophyllum alterniflorum, Utricularia vulgaris* agg., *Callitriche bruttia, Elatine hexandra, Callitriche hermaphroditica* and *Apium inundatum.*

Sand.

Dense *Potamogeton perfoliatus* and *Potamogeton gramineus.*

Sand and silt.

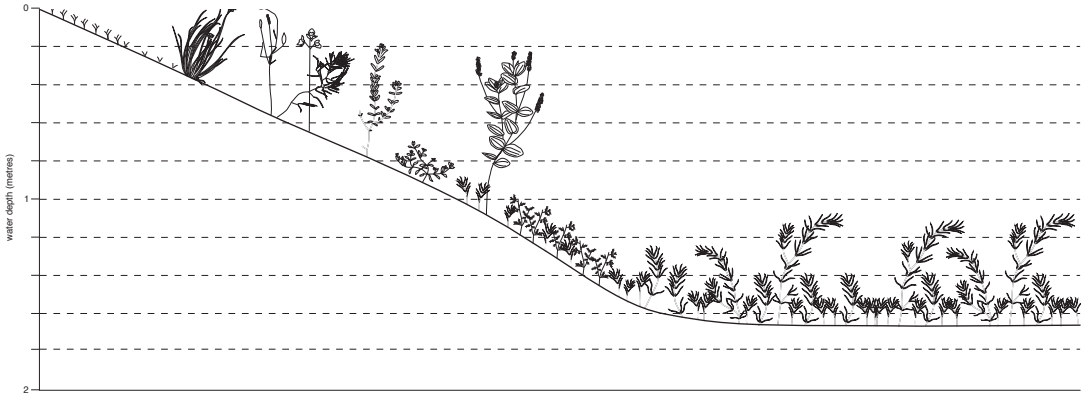
Dense *N. flexilis* (~70% cover).

Sand and silt.

Sparse *Potamogeton berchtoldii.*

Silt.

Loch Gearraidh Mhic Iain



Litorella uniflora, Lobelia dortmana and *Subularia aquatica.*

Sand and silt.

Very species rich zone, including *Potamogeton gramineus, Utricularia vulgaris* agg., *Callitriche bruttia* and *Elatine hexandra.*

Silt.

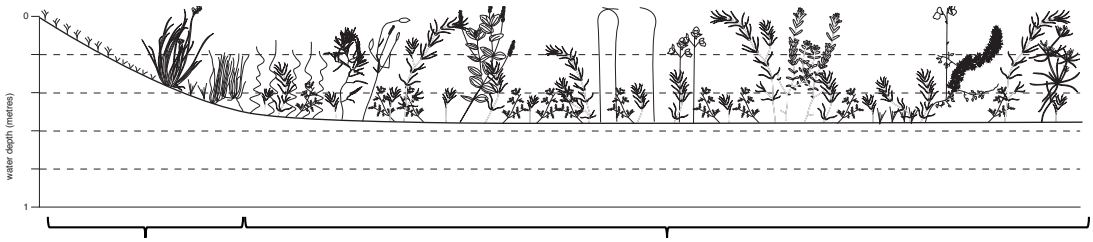
***N. flexilis* (~60% cover)** growing under dense canopy of *Myriophyllum alterniflorum.* Occasional *Potamogeton perfoliatus.*

Silt.

Dense *N. flexilis* (up to 100% cover).

Silt.

Loch a'Phuirt-Ruaidh



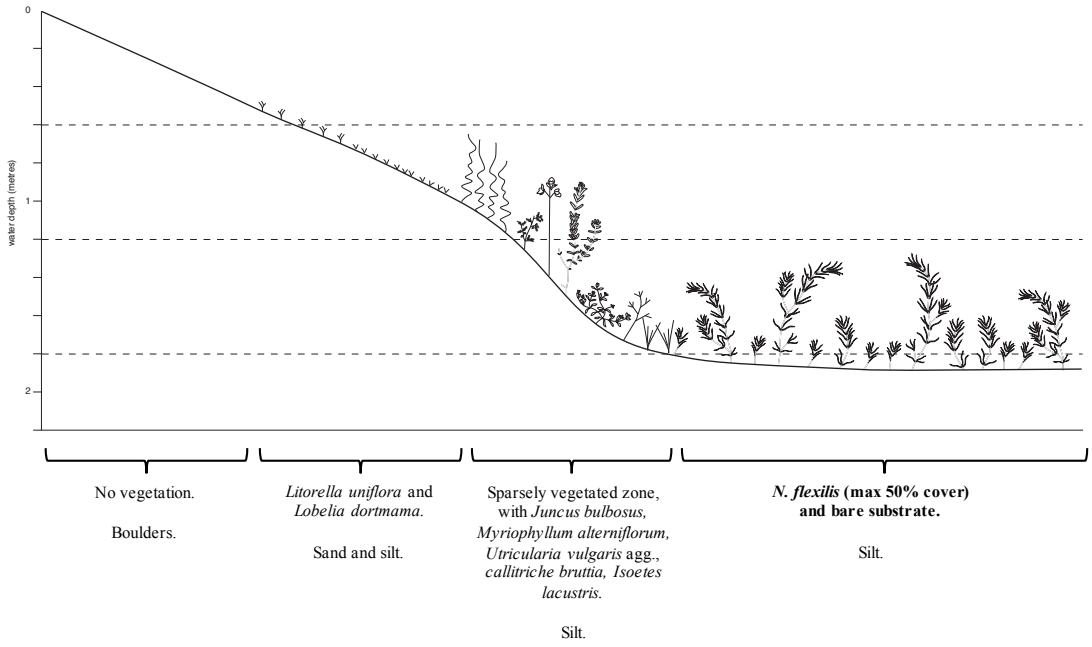
Litorella uniflora, Lobelia dortmana, Subularia aquatica, Isoetes lacustris and *Pillularia globulifera.*

Silt.

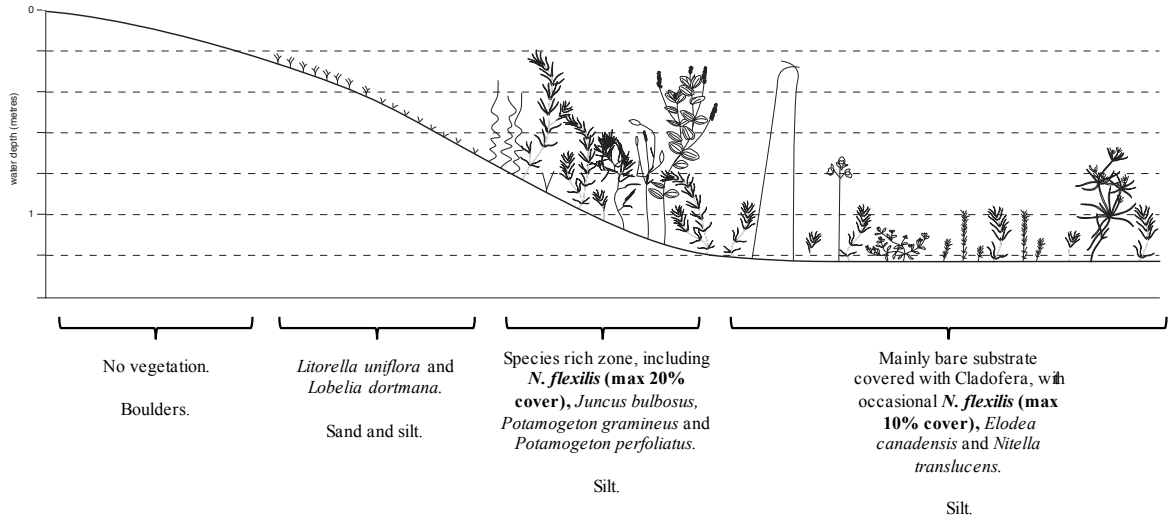
Very species rich zone, including *Juncus bulbosis, Potamogeton gramineus, Potamogeton perfoliatus, Sparganium angustifolium, Utricularia vulgaris* agg., *Callitriche bruttia* and *Nitella translucens.* ***N. flexilis* growing throughout (max 25% cover),** often under canopy of Myriophyllum alterniflorum.

Silt.

Loch na Eilean 3



Loch an Eilean 1 and 2



Mid Loch Ollay 1

Litorella uniflora.

Cobbles, pebbles and sand.

***N. flexilis* (max 15% cover)** interspersed with *Juncus bulbosus, Isoetes echinispera, Myriophyllum alterniflorum* and *Potamogeton x nitens.*

Sand and silt.

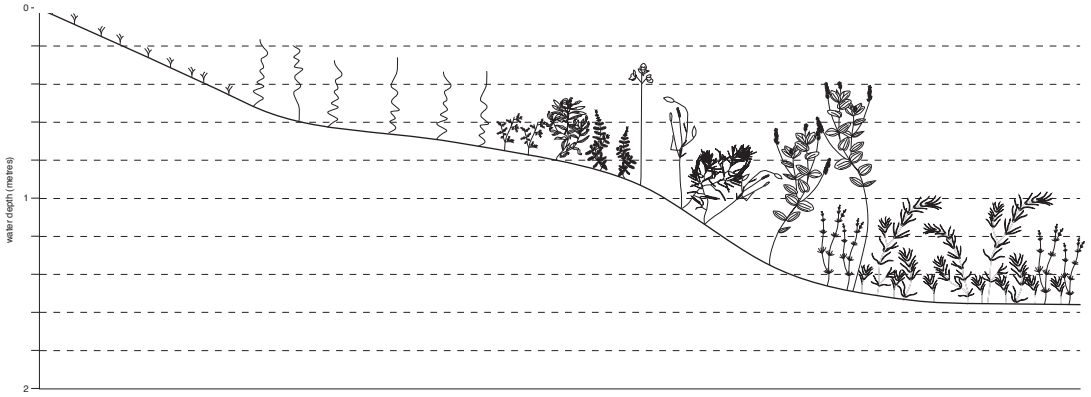
Potamogeton gramineus, Potamogeton perfoliatus, Potamogeton praelongus, Potamogeton x nitens.

Silt.

Dense *Sparganium angustofolium* and *Potamogeton natans.*

Silt.

Mid Loch Ollay 2



Litorella uniflora.

Cobbles, pebbles and sand.

Sparse *Juncus bulbosus.*

Samd and silt.

Juncus bulbosus, Myriophyllum alerniflorum, Potamogeton nitens, Utricularis vulgaris agg..

Silt.

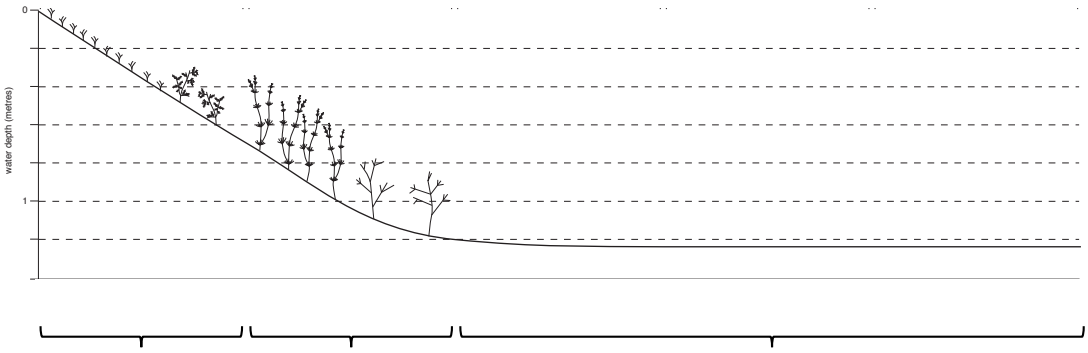
Dense *Potamogeton gramineus* and *Potamogeton perfoliatus,* interspersed with *Chara virgata.*

Silt.

Dense *N. flexilis* (70% cover) and occasional *Chara virgata..*

Silt.

Loch of Clunie T2



Litorella uniflora and occasional *Myriophyllum alterniflorum*.

Pebbles and cobbles.

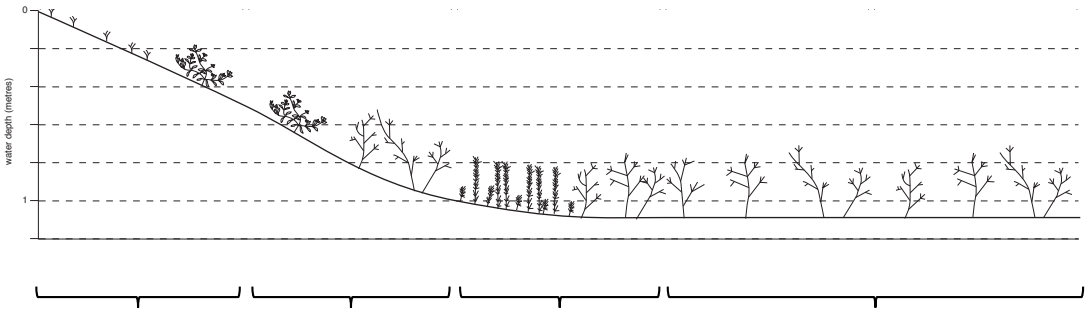
Chara virgata and *Nitella flexilis* agg.

Fine silt.

No vegetation.

Fine silt.

Loch of Butterstone 1



Litorella uniflora and *Elatine hexandra*.

Silt.

Occasional *Nitella flexilis* agg. and *Elatine hexandra*.

Silt.

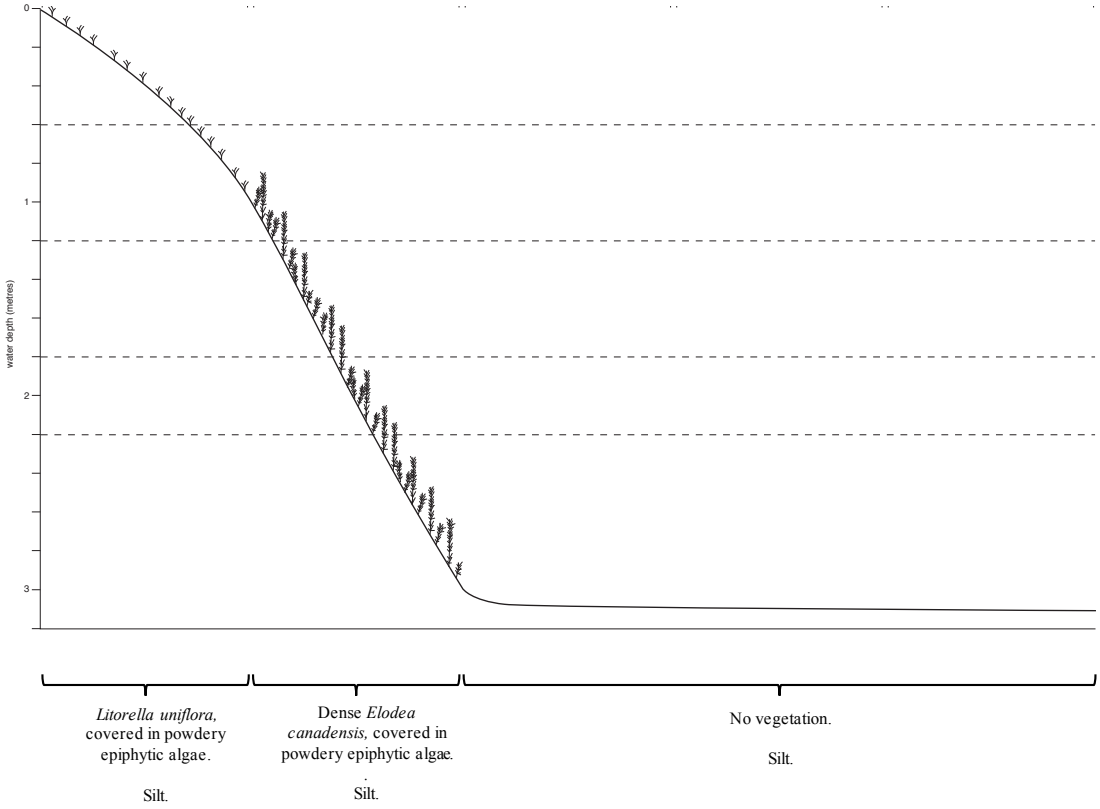
Dense *Elodea canadensis*.

Silt.

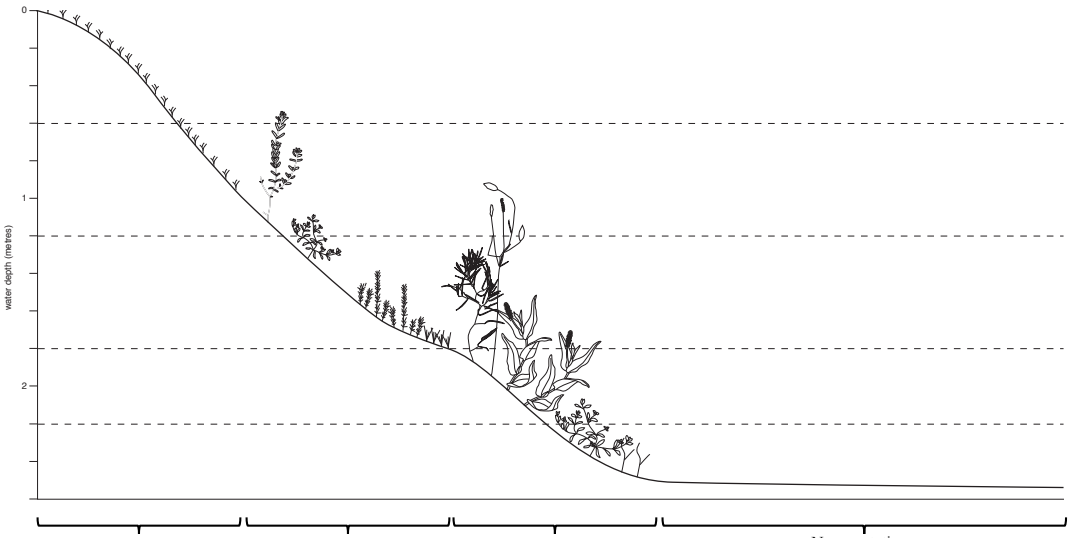
Nitella flexilis agg.

Silt.

Loch of Craiglush



Loch of Lowes



Litorella uniflora, covered in powdery epiphytic algae.

Silt.

Elodea canadensis interspersed with *Elatine hexandra, Callitriche bruttia* and *Isoetes lacustris,* covered in powdery epiphytic algae.

Silt.

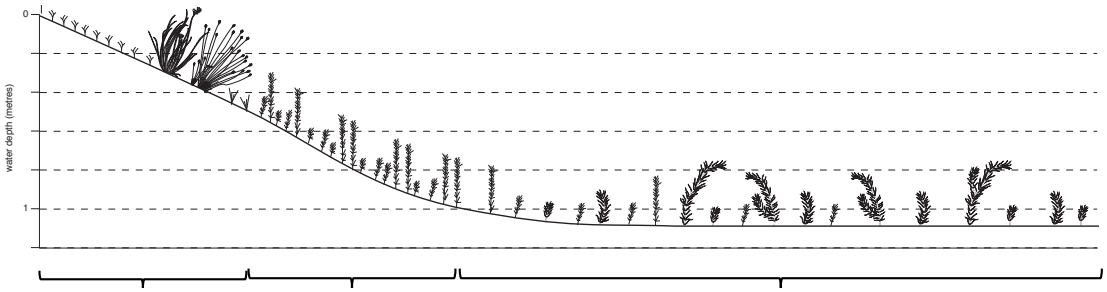
Potamogeton gramineus, Potamogeton praelongus and *Potamogeton obtusifolius,* occasional *Elatine hexandra*.

Silt.

No vegetation.

Silt.

Loch of Marlee 3



Litorella uniflora, Subularia aquatica, Eleocharis acicularis and *Isoetes lacustris*.

Cobbles, pebbles and silt.

Dense *Elodea canadensis*.

Silt.

Very occasional *Elodea canadensis* and *Callitriche hermaphroditica,* mostly bare substrate.

Silt.

Appendix 6: Full lists of macrophtye species found in each TWINSPAN division and subgroup

Division 1

Subgroup a	**Subgroup b**	**Subgroup g**
Alismataceae sp.	*Alnus glutinosa*	*Calitriche hermaphroditica*
Calitriche hermaphroditica	*Chara virgata*	*Callitriche brutia*
Carex rostrata	*Eleocharis acicularis*	*Callitriche hamulata*
Chara aspera	*Elodea canadensis*	*Chara aspera*
Chara virgata	*Isoetes echinospera*	*Chara virgata*
Elatine hexandra	*Isoetes lacustris*	*Elatine hexandra*
Elatine hydropiper	*Juncus bulbosis*	*Eleocharis acicularis*
Eleocharis acicularis	*Littorella uniflora*	*Elodea canadensis*
Eleocharis palustris	*Lobelia dortmana*	*Fontinalis antipyretica*
Elodea canadensis	*Myriophyllum alterniflorum*	*Isoetes lacustris*
Equisetum fluviatile	*Nitella translucens*	*Littorella uniflora*
Fontinalis antipyretica	*Nuphar lutea*	*Lythrum portula*
Isoetes lacustris	*Phragmites australis*	*Myriophyllum alterniflorum*
Lemna triscula	*Sparganium angustifolium*	*Nitella flexilis agg*
Littorella uniflora	*Sponge*	*Nitella translucens*
Lobelia dortmana		*Nuphar lutea*
Menyanthes trifoliata		*Persecaria amphibia*
Myriophyllum alterniflorum		*Potamogeton berchtoldii*
Nitella flexilis agg		*Potamogeton gramineus*
Nitella translucens		*Potamogeton natans*
Nostoc		*Potamogeton obtusifolius*
Nuphar lutea		*Potamogeton x nitens*
Nuphar x spenneriana		*Salix cinerea*
Phalaris arundinacea		*Sparganium erectum*
Phragmites australis		*Sponge*
Potamogeton berchtoldii		*Subularia aquatica*
Potamogeton crispus		*Zannichellia palustris*
Potamogeton gramineus		
Potamogeton obtusifolius		
Potamogeton perfoliatus		

Potamogeton pusillus
Potamogeton x nitens
Ranunculus aquatilis agg.
Salix cinerea
Solanum dulcamara
Subularia aquatica

Division 2

Subgroup c	**Subgroup d**	**Subgroup e**
Apium inundatum	*Agrostis stolonifera*	*Baldellia ranunculoides*
Baldellia ranunculoides	*Apium inundatum*	*Calitriche hermaphroditica*
Blindia acuta	*Baldellia ranunculoides*	*Calliergon cuspidatum*
Calliergon cuspidatum	*Calitriche hermaphroditica*	*Callitriche brutia var. hamulata*
Callitriche brutia var. hamulata	*Calliergon cuspidatum*	*Callitriche hamulata*
Carex nigra	*Callitriche brutia*	*Carex rostrate*
Carex rostrata	*Callitriche sp*	*Chara aspera*
Chara aspera	*Caltha palustris*	*Chara sp*
Chara sp	*Carex lepidocarpa*	*Chara virgate*
Chara virgata	*Carex nigra*	*Cladophora sp*
Eleocharis multicaulis	*Carex panicea*	*Drepanocladus sp.*
Eleocharis multicaulis	*Carex rostrata*	*Elatine hexandra*
Eleocharis palustris	*Chara virgata*	*Elatine sp*
Eleogiton fluitans	*Drepanocladus sp.*	*Eleocharis multicaulis*
Equisetum fluviatile	*Elatine hexandra*	*Eleocharis palustris*
Eriophorum angustifolium	*Eleocharis multicaulis*	*Eleogiton fluitans*
Fontinalis antipyretica	*Eleocharis palustris*	*Elodea canadensis*
Hydrocotyle vulgaris	*Eleogiton fluitans*	*Equisetum fluviatile*
Iris psuedacorus	*Equisetum fluviatile*	*Fontinalis antipyretica*
Isoetes echinospera	*Eriophorum angustifolium*	*Glyceria maxima*
Isoetes sp.	*Fontinalis antipyretica*	*Hydrocotyle vulgaris*
Juncus bulbosis	*Glyceria fluitans*	*Isoetes echinospera*
Littorella uniflora	*Hydrocotyle vulgaris*	*Isoetes lacustris*
Lobelia dortmana	*Iris psuedacorus*	*Isoetes sp.*
Menyanthes trifoliata	*Isoetes echinospera*	*Juncus bulbosis*

Molinia caerulea
Myrica gale
Myriophyllum alterniflorum
Najas flexilis
Nitella flexilis agg
Nitella translucens
Nymphaea alba
Persecaria amphibia
Phragmites australis
Potamogeton berchtoldii
Potamogeton gramineus
Potamogeton natans
Potamogeton perfoliatus
Potamogeton polygonifolius
Potamogeton pusillus
Potamogeton sp
Potentilla palustrus
Ranunculus flammula
Scorpidium scorpiodes
Sparganium angustifolium
Sparganium erectum
Sphagnum sp
Sponge
Utricularia intermedia agg
Utricularia minor
Utricularia stygia
Utricularia vulgaris agg

Isoetes lacustris
Isoetes sp.
Juncus articulatus
Juncus bulbosis
Lemna minor
Littorella uniflora
Lobelia dortmana
Mentha aquatica
Menyanthes trifoliata
Moss sp
Myosotis laxa
Myriophyllum alterniflorum
Najas flexilis
Nitella flexilis agg
Nitella translucens
Nymphaea alba
Pilularia globulifera
Potamogeton berchtoldii
Potamogeton gramineus
Potamogeton natans
Potamogeton perfoliatus
Potamogeton pusillus
Potamogeton x nitens
Potentilla palustrus
Ranunculus flammula
Rorippa nasturtium-aquaticum agg.
Scorpidium scorpiodes
Sparganium angustifolium
Sponge
Subularia aquatica
Utricularia minor
Utricularia stygia

Lemna minor
Lemna triscula
Littorella uniflora
Lobelia dortmana
Menyanthes trifoliata
Myriophyllum alterniflorum
Myriophyllum spicatum
Najas flexilis
Nitella flexilis agg
Nitella sp
Nostoc
Nuphar lutea
Nuphar pumila
Nymphaea alba
Persecaria amphibia
Phragmites australis
Pilularia globulifera
Potamogeton berchtoldii
Potamogeton filiformis
Potamogeton friesii
Potamogeton gramineus
Potamogeton lucens
Potamogeton natans
Potamogeton pectinatus
Potamogeton perfoliatus
Potamogeton praelongus
Potamogeton pusillus
Potamogeton sp
Potamogeton x nitens
Potentilla palustrus
Ranunculus flammula
Schoenoplectus lacustris

Utricularia vulgaris agg	*Scorpidium scorpiodes*
	Sparganium angustifolium
	Sparganium natans
	Subularia aquatica
	Utricularia intermedia agg
	Utricularia sp
	Utricularia vulgaris agg
	Zannichellia palustris

Division 3

Subgroup f	**Subgroup j**
Calitriche hermaphroditica	*Eleocharis acicularis*
Callitriche platycarpa	*Equisetum fluviatile*
Chara sp	*Isoetes lacustris*
Elodea canadensis	*Juncus bulbosis*
Isoetes echinospera	*Littorella uniflora*
Isoetes lacustris	*Lobelia dortmana*
Juncus bulbosis	*Myriophyllum alterniflorum*
Littorella uniflora	*Najas flexilis*
Lobelia dortmana	*Nitella conferacaea*
Myriophyllum alterniflorum	*Nitella flexilis agg*
Myriophyllum spicatum	*Nitella translucens*
Najas flexilis	*Potamogeton berchtoldii*
Nitella flexilis agg	*Potamogeton gramineus*
Nitella translucens	*Potamogeton natans*
Nuphar lutea	*Potamogeton pectinatus*
Nymphaea alba	*Potamogeton perfoliatus*
Phragmites australis	*Potamogeton praelongus*
Potamogeton berchtoldii	*Sparganium angustifolium*
Potamogeton gramineus	*Utricularia vulgaris agg*
Potamogeton natans	

Potamogeton perfoliatus
Schoenoplectus lacustris
Sparganium angustifolium
Utricularia sp

Division 4

Subgroup h	**Subgroup i**
Baldellia ranunculoides	*Apium inundatum*
Brachythecium rutabulum	*Baldellia ranunculoides*
Calitriche hermaphroditica	*Calitriche hermaphroditica*
Calliergon cuspidatum	*Calliergon cuspidatum*
Callitriche hamulata	*Caltha palustris*
Chara aspera	*Chara aspera*
Chara curta	*Chara contraria*
Chara hispida	*Chara hispida*
Chara sp	*Chara rudis*
Chara virgata	*Chara virgata*
Eleocharis palustris	*Drepanocladus sp.*
Elodea nuttallii	*Eleocharis multicaulis*
Equisetum fluviatile	*Eleocharis palustris*
Fontinalis antipyretica	*Elodea nuttallii*
Hydrocotyle vulgaris	*Equisetum fluviatile*
Isoetes echinospera	*Fontinalis antipyretica*
Juncus articulatus	*Littorella uniflora*
Littorella uniflora	*Myriophyllum alterniflorum*
Menyanthes trifoliata	*Myriophyllum spicatum*
Moss sp	*Nostoc*
Myriophyllum alterniflorum	*Persecaria amphibia*
Myriophyllum sp	*Phragmites australis*
Najas flexilis	*Potamogeton filiformis*
Nitella flexilis agg	*Potamogeton gramineus*
Potamogeton berchtoldii	*Potamogeton natans*
Potamogeton filiformis	*Potamogeton pectinatus*
Potamogeton gramineus	*Potamogeton perfoliatus*
Potamogeton natans	*Potamogeton pusillus*
Potamogeton pectinatus	*Potamogeton sp*
Potamogeton perfoliatus	*Potamogeton x nitens*

Potamogeton pusillus
Potamogeton rutilis
Potamogeton x nitens
Sponge
Zannichellia palustris
Scorpidium scorpiodes

Appendix 7: Macrofossils, cladocera and diatoms found in ESTH9 and ESTH1/7

Macrofossils	**Abbreviation**
N flexilis seeds	Nflex_seed
N flexilis spines	Nflex_spine
Daphnia ephippia	Daph
Trichoptera frontoclypeal apotomes	Trich_FA
Corixidae hemielytron	Cor_hem
Moss fragments	Moss
Nymphaea seed fragments	Nymp_seed
Root fragments	Root
Chidorideae carapaces	Chid
Trichoptera pupal case membranes	Trich_caselid
Potamogeton obtusifolius agg. leaf tips	Pobt_leaf
Corixidae tails	Cor_tail
Nymphaea trichlosclereids	Nymp_tri
Equisitum fluviatale stem diaphragm	Eflu
Crystotella statoblasts	Cryst
Utricularia vulgaris bladders	Uvulg_blad
Typha latifola seeds	Tlat
Potamogeton leaf fragments	Pot_leaf
Gleotrichia-like colony	Gleo
Potamogeton berchtolii leaf tips	Pberch_leaf
Mys. Pers.	Mpers
Snail operculum	Snail
Isoetes lacustris seeds	Ilac
Potamogeton perfoliatus seeds	Pperf_seed
Nitella oospores	Nit
Chara oospores	Chara
Nuphar lutea seed fragments	Nlut_seed
Juncus bulbosus seeds	Jbulb
Cereodaphnia ephippia	Cdaph
Unidentified bryozoan	Ubry
Trichoptera pupal cases	Trich_case
Callitriche hermaphroditica seeds	Cherm
Betula pubescens seeds	Bpub
Utricularia vulgaris seeds	Uvulg_seed

Lobelia dortmana seeds	Ldort
Potamogeton berchtoldii seeds	Pberch_seed

Cladocera	**Abbreviation**
Daphnia longispina	D_long
Daphnia pulex	D_pul
Daphnia filter	D_filt
Bosmina coreogonis	B_cor
Bosmina longirostris	B_longr
Bosmina longispina	B_longs
Sida	S_crys
Acroperus harpae	A_harp
Alona affinis	A_aff
Alona guttata/rectangularis	A_gutt
Alona quadrangularis	A_quad
Alonella exigua	A_exig
Alonella exisa	A_exis
Alonella nana	A_nana
Camptocercus rectirostris	C_rect
Chydorus piger	Ch_pig
Cydorus sphericus	Ch_sph
Eurycercus	Eury
Graptoloberis	Grap
Leydigia leydigi	Leyd
Pleuroxis	Pleu
Cereodaphnia ephippia	Cdaph_eph
Daphnia longispina ephippia	Dlong_eph
D. pulex ephippia	Dpul_eph
Bosmina longirostris ephippia	Blong_eph
Camptocercus rectirostris ephippia	Crect_eph
C. sphericus ephippia	Chsph_eph

Diatoms	**Abbreviation**
Achnanthes lanceolata	
Achnanthes linearis	
Achnanthes clevei var clevei	
Achnanthes minutissima var minutissima	A_min
Achnanthes pusilla var pusilla	
Achnanthes pusilla var pusilla	

Achnanthes levanderi
Achnanthes laevis
Achnanthes subatomoides
Achnanthes sp.
Amphipleura pellucida
Amphora fogediana
Amphora libyca
Amphora pediculus
Amphora inariensis
Amphora sp.
Anomoeoneis brachysira
Asterionella formosa var formosa A_form
Brachysira vitrea
Caloneis schumanniana
Caloneis sp.
Cocconeis placentula var placentula
Cocconeis placentula var euglypta
Cocconeis placentula var lineata
Cocconeis pediculus
Cocconeis diminuta
Cyclostephanos invisitatus
Cyclostephanos tholiformis
Cymbella sinuata
Cymbella sinuata
Cymbella microcephala
Cymbella cistula var cistula
Cymbella cesatii var cesatii
Cymbella amphicephala var amphicephala
Cymbella gracilis
Cymbella affinis
Cymbella minuta var minuta
Cymbella mesiana
Cymbella silesiaca
Cymbella sp.
Cyclotella comta var comta
Cyclotella pseudostelligera
Cyclotella meneghiniana var meneghiniana
Cyclotella stelligera

Cyclotella kuetzingiana var kuetzingiana	
Cyclotella glomerata	
Cyclotella ocellata	
Cyclotella comensis	C_com
Cyclotella radiosa	C_rad
Cyclotella unipunctata	
Cyclotella cyclopuncta	
Cyclotella kisselevii	
Cyclotella sp.	
Denticula tenuis var tenuis	
Denticula tenuis var crassula	
Diatoma elongatum	
Diatoma tenue var tenue	
Diatoma sp.	
Diploneis oblongella var oblongella	
Diploneis elliptica var elliptica	
Diploneis sp.	
Epithemia sp.	
Eunotia pectinalis var ventralis	
Eunotia praerupta var praerupta	
Eunotia exigua var exigua	
Eunotia arcus var arcus	
Eunotia incisa	
Eunotia bilunaris	
Eunotia bilunaris var mucophila	
Eunotia implicata	
Eunotia sp.	
Fragilaria pinnata var pinnata	
Fragilaria construens var construens	
Fragilaria construens var binodis	
Fragilaria construens var venter	
Fragilaria virescens var virescens	
Fragilaria virescens var exigua	
Fragilaria brevistriata var brevistriata	
Fragilaria crotonensis	F_crot
Fragilaria capucina var capucina	
Fragilaria capucina var mesolepta	
Fragilaria capucina var austriaca	

Fragilaria capucina var perminuta
Fragilaria elliptica
Fragilaria intermedia
Fragilaria intermedia var continua
Fragilaria parasitica
Fragilaria parasitica var subconstricta
Fragilaria tenera
Fragilaria incognita
Fragilaria incognita
Fragilaria incognita
Fragilaria sp.
Frustulia sp.
Gomphonema olivaceum
Gomphonema angustatum var angustatum
Gomphonema gracile
Gomphonema acuminatum var trigonocephalum

Gomphonema constrictum
Gomphonema parvulum var parvulum
Gomphonema truncatum var truncatum
Gomphonema clevei
Gomphonema clavatum
Gomphonema minutum
Gomphonema sp.
Hannaea arcus var arcus
Meridion circulare var circulare
Meridion circulare var constrictum
Navicula radiosa var radiosa
Navicula hungarica
Navicula seminulum
Navicula cryptocephala var cryptocephala
Navicula cryptocephala var veneta
Navicula rhyncocephala var rhyncocephala
Navicula lanceolata
Navicula pseudoscutiformis
Navicula pupula var pupula
Navicula cincta
Navicula gregaria

Navicula mutica var mutica
Navicula viridula var viridula
Navicula scutelloides
Navicula minima var minima
Navicula graciloides
Navicula trivialis
Navicula pseudolanceolata
Navicula atomus
Navicula subminuscula
Navicula vitabunda
Navicula helensis
Navicula minusculoides
Navicula tenera
Navicula vitiosa
Navicula ordinaria
Navicula ordinaria
Navicula sp.
Neidium sp.
Nitzschia fonticola
Nitzschia perminuta
Nitzschia frustulum
Nitzschia palea var palea
Nitzschia amphibia var amphibia
Nitzschia dissipata
Nitzschia recta
Nitzschia microcephala
Nitzschia linearis var linearis
Nitzschia linearis var tenuis
Nitzschia inconspicua
Nitzschia tubicola
Nitzschia epithemioides
Nitzschia subacicularis
Nitzschia lacuum
Nitzschia bacillum
Nitzschia lancettula
Nitzschia sp.
Pinnularia interrupta
Pinnularia major var major

Pinnularia microstauron	
Pinnularia subcapitata var subcapitata	
Pinnularia obscura	
Pinnularia sp.	
Sellaphora pupula var pupula	
Stephanodiscus hantzschii	S_hanz
Stephanodiscus alpinus	
Stephanodiscus parvus	
Stephanodiscus medius	
Stephanodiscus sp.	
Stauroneis phoenicenteron var phoenicenteron	
Stauroneis sp.	
Staurosira construens var venter	
Surirella sp.	
Aulacoseira ambigua	
Aulacoseira granulata var angustissima	A_gran_ang
Aulacoseira granulata var muzzanensis	A_gran_muzz
Aulacoseira islandicaislandica	A_isla
Aulacoseira subarctica	A_sub
Aulacoseira sp.	
Aulacoseira sp.	
Synedra ulna var ulna	
Synedra acus var acus	
Synedra acus var radians	
Synedra nana	
Synedra minuscula	
Tabellaria flocculosa var flocculosa	T_floc
Thalassiosira sp.	

Appendix 8: Macrofossils and cladocera found in CRAIB

Macrofossils	**Abbreviation**
N. flexilis seeds	Nflex_seed
N. flexilis spines	Nflex_spine
Daphia ephippia	Daph_eph
Trichoptera frontoclypeal apotomes	Tri_Fa
Corixidae hemielytron	Cor_hem
Spagnum	Sphag
Nymphaea seed fragments	Nymp_seed
Root fragments	Root
Chidoridae carapaces	Chid
Trichoptera pupal case membrane	Tri_caselid
Corixidae tails	Cor_tail
Nymphaea triclosclerids	Nymp_tri
Crystatella statoblasts	Cryst
Potamogeton leaf fragments	Pot_leaf
Mys. Pers.	Mpers
Isoetes lacustris seeds	Ilac
Nitella oospores	Nit
Chara oospores	Chara
Nuphar lutea seed fragments	Nlut_seed
Unidentified bryozoan	Ubry
Trichoptera pupal cases	Tri_case
Surirella sp.	Suri
Pediastrum	Ped
Juncus bulbosis seeds	Jbulb
Cereodaphnia ephippia	Cdaph_eph
Unidentified oligochaete	Uolig
Snail operculum	Snail
Potamogeton alpinis seeds	Palp_seed
Potamogeton gramineus seeds	Pgram_seed
Potamogeton perfoliatus seeds	Pperf_seed
Potamogeton berchtoldii seeds	Pberch_seed
Persecaria seeds	Perc
Betula pubescens seeds	Bpub
Callitriche bruttia seeds	Cbrut
Potamogeton natans seeds	Pnat_seed
Lobelia dortmana seeds	Ldort

Cladocera	**Abbreviation**
Daphnia longispina	Dlong
Daphnia pulex	Dpul
Bosmina coregonis	Bcor
Bosmina longispina	Blongs
Bosmina longirostris	Blongr
Latona setifera	Lset
Sida crystallina	Scrys
Acoroperus harpae	Aharp
Alona affinis	Aaff
Alona guttata/rectangularis	Agutt
Alona guttata/rectangularis var tuberculosis	Agutt_tub
Alona quadrangularis	Aquad
Alonella exigua	Aexig
Alonella exisa	Aexis
Alonella nana	Anana
Alonopsis elongata	Aelon
Camptocercus rectirostris	Crect
Chydorus piger	Chpig
Chydorus sphericus	Chsph
Chydorus sphericus var coelatus	Chsph_coe
Eurycerus spp.	Eury
Graptoloberis	Grap
Leydigia leydigi	Leyd
Pleuroxis	Pleu
Cereodaphnia ephippia	Cdaph_eph
Daphnia longispina ephippia	Dlong_eph
Bosmina coregoni ephippia	Bcor_eph

Appendix 9: Priority sites for establishing the current status of *N. flexilis*

Site name	Grid reference	County	Date of first record	Date of last record	Date of last known survey
Mainland Scotland					
Fingask Loch	NO165431	Perthshire	1877	2006	2006
Inner Isles					
Loch Poit na h-I	NM314227	Mull	1921	1999	1999
Western Isles					
Loch Eilean a' Ghille-Ruaidh	NF769366	South Uist	1983	1999	1999
Loch Altabrug	NF745340	South Uist	1983	2000	2000
Loch nam Faoileann	NF752210	South Uist	1995	2000	2000
Ireland					
Clooney Lough	G79	Donegal	1939	2002	2002
Lough Mullaghderg	B71	Donegal	1919	2002	2002
Lough Nagreaney	C1441	Donegal	1990	2002	2002
Lough Shannagh	C2045	Donegal	1989	2002	2002
Loch an Phoirt	C0034	Donegal	1989	2006	2006
Roundstone	L73	Galway		1896	1896
Roundstone	L73	Galway	1833	1907	1907
Lough Nalawney	L690415	Galway	1977	1995	1995
Loch an Chaolaigh	L804307	Galway	1996	2004	2004
Loch na gCaor	L73	Galway	1974	2004	2004
Lough Barnahallia	L593556	Galway	1975	2004	2004
Lough Cregduff	L73	Galway	1852	2004	2004
Lough Derrywaking	L6748	Galway	1989	2004	2004

Lough Emlaghnacourty	L654459	Galway	1999	2004	2004
Lough Keeraun	L784315	Galway	1990	2004	2004
Lough Lehanagh	L85	Galway	1975	2004	2004
Lough Natawnymore	L92	Galway	1990	2004	2004
Lough Rusheenduff	L66	Galway	1935	2004	2004
Lough Truskan	L808305	Galway	1990	2004	2004
Lough Tully	L692616	Galway	1978	2004	2004
Seanchrois	L632430	Galway	1999	2005	2005
Ards Peninsula	L7531	Galway	1991	1991	1991
Lough Dahybaun	G01	Mayo	1977	2004	2004
Lough Nageltia	M114785	Mayo	2004	2004	2004
Lough Nahaltora	L793741	Mayo	2000	2004	2004